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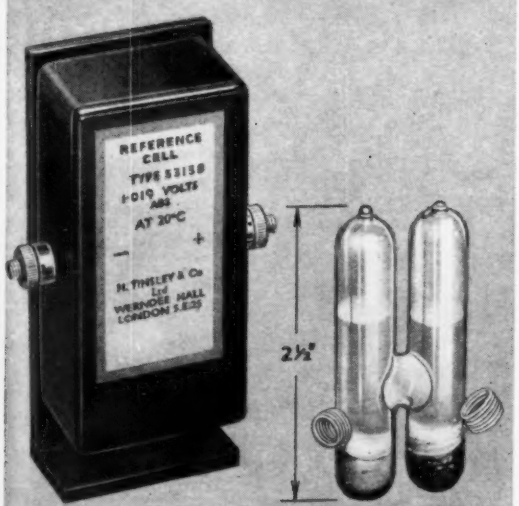

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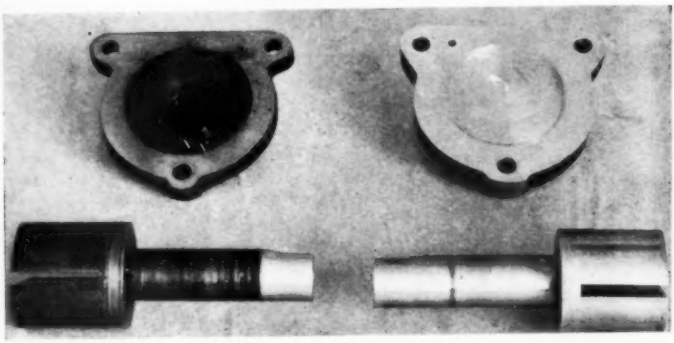

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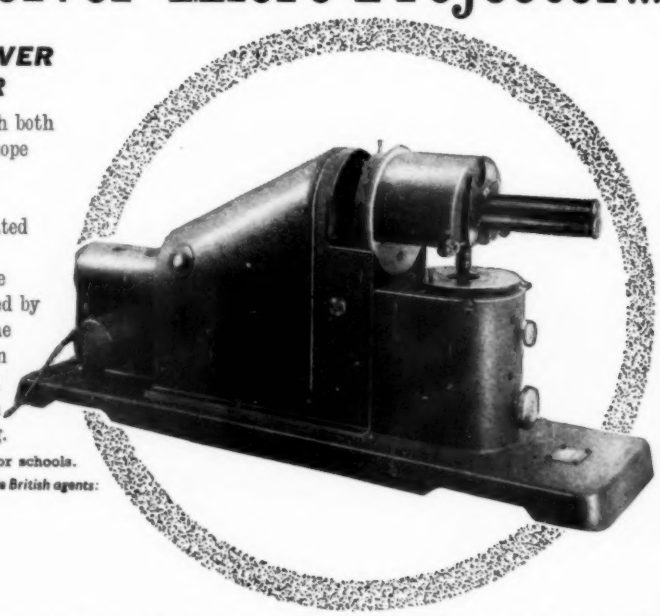

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NATURE

No. 4735 SATURDAY, JULY 30, 1960 Vol. 187

CONTENTS

	Page
Scientist and Technologist in Administration	353
Nullius in verba. By Dr. William P. D. Wightman	356
Genetics for Beginners. By Miss Wilma George	357
Identification of Blood-Group Antigens. By Dr. R. Drummond	357
Evolution of Modern Education. By Dr. James L. Henderson	357
Nuclear Magnetic Resonance. By Dr. L. M. Jackman	358
Principles and Practice of Television Engineering. By Dr. R. L. Smith-Rose, C.B.E.	358
Unification of Analytical Approaches to Engineering Problems. By Prof. J. Greig	359
Rubber Technology. By Dr. W. F. Watson	359
Marine Biological Reports from Denmark. By Dr. A. J. Southward	360
Westfield College (University of London)	361
Control of Pre-Natal Growth in Mammals. By Dr. Anne McLaren and Dr. Donald Michie	363
Nature of the Hæm-binding Groups in Native and Denatured Hæmoglobin and Myoglobin. By Dr. Joan Keilin	365
News and Views	371
Doctor Ruggles Gates and the Aboriginal Australian. By Prof. A. A. Abbie	375
Herbicides and the Soil	376
Colloid and Polymer Science	378
Effectiveness in Industry	378
Health Services in the U.S.S.R.	379
Biological Effects of Atomic Radiation. By Dr. C. Auerbach, F.R.S.	380
Modernization of the Stockholm Cyclotron	381
Daily Food Consumption by South African University Students	381
Distribution of Atmospheric Ozone; a Preliminary Analysis of some International Geophysical Year Observations. By J. MacDowall	382
Theory of Auroral Structures. By E. R. Harrison	383
Infra-red Spectra of Amino-Acids and Related Substances in Water. By Prof. Frank S. Parker and Prof. Donald M. Kirschenbaum	386
A Hypothesis of Contraction in Striated Muscle. By Dr. M. Spencer and Dr. C. R. Worthington	388
Melanin Granules and Mitochondria. By Dr. Ruth V. Baker, M. S. C. Birbeck, Dr. H. Blaschko, Prof. T. B. Fitzpatrick and Dr. M. Seiji	392
Critical Energy Barriers in the Excretion of Sodium. By Prof. E. J. Conway, F.R.S.	394
Recent Scientific and Technical Books	Supp. vi

(*Continued overleaf*)

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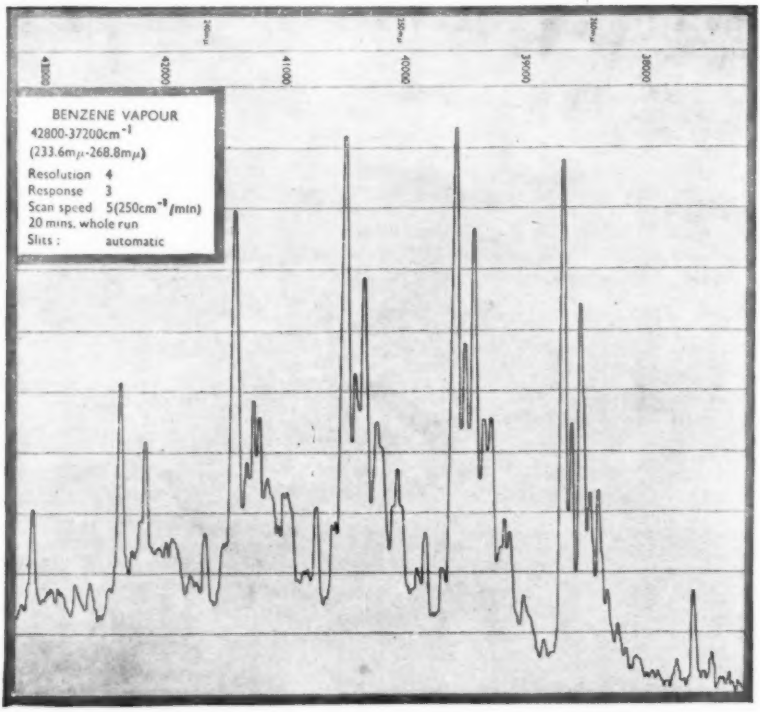

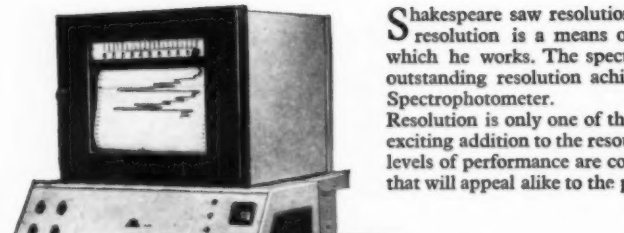

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LETTERS TO THE EDITORS

Page

ASTROPHYSICS

Local Increase of Intensity of Cosmic Radiation.—J.-P. Legrand and Mrs. A. Helary . . 397

RADIOPHYSICS

A Highly Directive Rotating Array for 16 Mc./s.—Prof. J. A. Thomas and Dr. R. W. E. McNicol 398

Radar Determination of the Scattering Properties of the Moon.—Dr. Fred B. Daniels . . 399

GEOPHYSICS

Anomalous Leads from a Selected Geological Environment in West-Central New Mexico.—William F. Slawson and Carl F. Austin . . 400

PHYSICS

A Method for the Routine Measurement of Particle Shape Factors in the Sieve Range.—Dr. Colin C. Harris; D. Howitt 401

Gallium Arsenide as a Semi-insulator.—J. W. Allen 403

Gallium Arsenide for γ-Ray Spectroscopy.—W. R. Harding, C. Hilsum, M. E. Moncaster, D. C. Northrop and Dr. O. Simpson . . 405

ENGINEERING

Pump Fluids for Higher Vacuums.—Kenneth Hickman 405

METALLURGY

The Sulphide Phase in Some Iron Meteorites.—Dr. H. J. Axon 406

A New Phase in a Silicon bearing 17 per cent Chromium, 8 per cent Nickel and 1 per cent Niobium Steel.—M. J. O'Hara, D. Wilkinson and R. T. Allsop 407

CHEMISTRY

Mass Spectrometric Investigation of the Vaporization of Sulphides and the Dissociation Energy of S_2.—R. Colin, Prof. P. Goldfinger and M. Jeunehomme 408

Ionization and Attachment Coefficients in *n*-Alkyl Chloride Gases.—J. C. Devins and O. H. LeBlanc, jun. 409

Homogeneous Synthesis of Isotactic Polystyrene using *n*-Butyllithium Initiator.—Dr. R. J. Kern 410

BIOCHEMISTRY

Presence of Neutral Carbohydrates in Human Aortic Tissue.—Dr. Sv. Bertelsen . . . 411

Organic Matrix of Tooth Enamel.—J. E. Eastoe 411

Starch-like Polysaccharide of *Streptococcus pyogenes*.—Dr. H. Baum and Dr. N. Crowley . 412

Ubiquinone (50) and Ubichromenol in *Aspergillus fumigatus*, Fresenius.—N. M. Packter and Dr. J. Glover 413

Bradykinin-releasing Factor from *Bothrops jararaca* venom.—Dr. Olga B. Henriques, Mina Fichman and W. T. Beraldo 414

Anti-Pneumococcus Type XIV Activity of Precipitins from *Ricinus communis* Seeds.—Lt.-Col. G. W. G. Bird 415

Enzymatic Synthesis of Formylglutamic Acid by a Plant Protein.—Ernest H. Byers, jun., and T. J. Bond 416

Crystallization of a Photosynthetic Reductase from a Green Plant.—Dr. R. Hill, F.R.S., and Fay Bendall 417

Page

PHYSIOLOGY

Differential Reactivity of Saliva from 'Tasters' and 'Non-Tasters' of 6-*n*-Propylthiouracil.—Frances Griffin and Prof. Roland Fischer . 417

Flight by Man-Power.—Dr. E. Guerra and Dr. B. Günther 419

Effect of Nitrate Ion on the Distribution of Sucrose in the Rat's Ventricle.—S. T. Taketa, C. G. Alexander and Prof. G. A. Feigen . . . 419

An Unidentified Pharmacologically Active Substance extracted from Cerebral Hemispheres.—P. F. L. Andrews, Shirley A. P. Price and Dr. G. B. West 420

Production of Muscular Weakness in Rats by a Creatine Analogue.—Coy D. Fitch and Prof. James S. Dinning 421

Damage to Liver induced by DL-Methionine-Ethylester.—I. Fehér, F. V. Selmeczi, K. Szalay and Dr. T. Kemény 421

Effect of Some Aromatic Hydroxy Compounds on the Phagocytic Activity of the Reticulo-Endothelial System.—Prof. T. Nicol, C. Druce and C. C. Ware 422

PATHOLOGY

Production of Tumours in *Rattus* (*Mastomys*) *natalensis* by Polyoma Virus.—Dr. Alan S. Rabson, William J. Branigan and Frances Y. Legallais 423

Hæmodynamic Aspects of Atherogenesis.—Wei Young 425

Anti-neoplastic Activity of Tetrahydro-1,3-oxazine Derivatives.—Prof. T. Urbański, D. Gürne, S. Ślopek, H. Mordarska and M. Mordarski 426

BIOLOGY

Fungitoxicity of Metal Ions.—Dr. E. Somers . 427

A Method for the Detection of Inhibition of Auxin Transport.—B. E. S. Gunning . . . 428

Maternal Inheritance and Interspecific Hybrids of the Liverwort *Sphaerocarpos*.—Dr. William T. Doyle 429

Significance of the Type of Host Plant Crop in Successful Biological Control of Insect Pests.—D. C. Lloyd 430

Diplodiscus Species Parasitic in the Snail *Paludina vivipara* Lm.—M. R. Honer 431

Effect of Pyruvate upon Nucleated and Enucleated Fragments of *Amoeba proteus*.—Dr. Edmund Guttes and Sophie Guttes . . 432

BACTERIOLOGY

Egg-Yolk Reactions of *Pseudomonas* Species.—Dr. A. T. Willis and G. Gowland . . . 432

Two Types of Catalase-like Activity in Lactic Acid Bacteria.—R. Whittenbury . . . 433

Production of Tuberculin Sensitivity.—Prof. Hubert Bloch and Albert A. Nordin . . 434

SOIL SCIENCE

Potassium-supplying Power of Some British Soils.—P. W. Arnold 436

STATISTICS

Analysis of Small Experiments.—P. Sprent . 437

Significance of the Difference between Two Means when the Population Variances may be Unequal.—John B. de V. Weir 438

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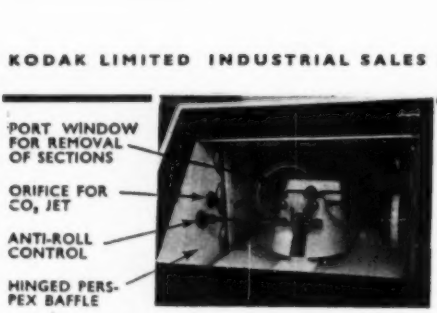

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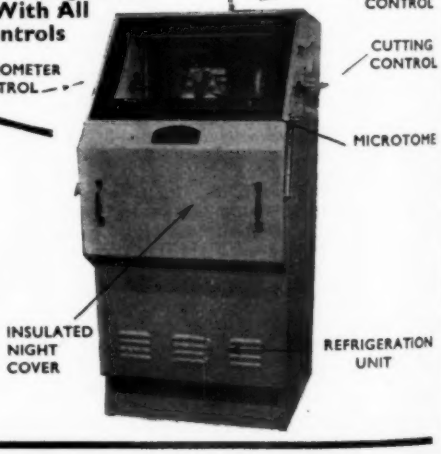

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SCIENTIST AND TECHNOLOGIST IN ADMINISTRATION

A DEPARTMENT of Science and Technology in the Federal Government of the United States, headed by a cabinet secretary and responsible for a considerable range of scientific and technological activities, has been proposed in several recent Senate bills and recommended in varying forms by administrators of such scientific standing as Dr. L. V. Berkner and Dr. W. R. Brode. Early in January 1960, these proposals were discussed by a group which included Dr. Berkner, Prof. A. H. Dupre, Mr. D. K. Price, Mr. Dael Wolfle and three others. The group agreed in advance to concentrate on the reasons for considering such a department, the alternative forms it might take, and their probable consequences for science and engineering, and for the ability of the Federal Government to carry out its responsibilities in these fields.

The report based on these discussions which Mr. Wolfle was asked to prepare and which has been published in *Science* of May 13 (131, 1407 ; 1960) has thus an interest for scientists and technologists generally and not simply for those concerned with the relations between government and science in the United States. It makes a genuine attempt to arrive at first principles, and the Group's findings and comments have considerable relevance to the functions of the Minister for Science in Britain and the organization for which he is responsible. The arguments in favour of a Department of Science and Technology are carefully examined, the implications are indicated, and sound criteria are defined which can be applied to any changes in the structure by which scientific advice is brought to bear on problems of government, no less in Great Britain than in the United States.

The first major criterion for judging an organization is its ability to get the assigned jobs done as well as possible, and here the American Group clearly has grave reservations as to how far the proposed Department would offer advantages over the existing arrangements. It does not accept the view that complete elimination of duplication is a valid objective, nor does it consider that co-ordination is necessarily more effective through a single department, as against proper communication between a number of independent departments. Efficient organization is essential, and this applies to any such new department and to existing departments or agencies the activities of which require co-ordination. Both principles are to be kept clearly in mind in considering the activities and interrelations for which the Minister for Science is responsible and also for some, like those in the fields of defence and of education, for which direct responsibility lies elsewhere. It is, in fact, precisely because some recent developments have raised doubts as to the operational effectiveness of the Defence Research Policy Committee and the efficiency of the organization of higher technological education under the control of the Minister of Education and the local authorities, that changes have been suggested for Britain.

Again, the Group rejects the argument that establishment of a Department of Science and Technology would increase financial support for such activities. Such support from public funds, it is rightly pointed out, must come from an increase in public understanding, though the Group accepts as probably sound, though not compelling, the argument that establishment of such a Department would enhance the status of science and technology as a government activity. Here again much depends on effective public relations, and it seems probable that if the existing official research councils in Britain are adequately financed and their activities effectively interpreted to the general public, the Minister for Science should be able to command sufficient public attention and prestige for the activities for which he is responsible. That undoubtedly comes first, for immediately science begins to affect policy, public understanding is imperative.

This appears clearly enough from the fourth criteria which the Group applies to the organization of scientific and technological activity—a capacity to promote the development of sound policy. Scientific and technical activities constitute one of the elements that must be considered in many aspects of broad national policy. At some point in the structure of government there must be means for bringing all the scientific and technological threads together, both from the point of view of establishing policy for research and development agencies and from that of the contributions these agencies make to national policy.

The Group is emphatic that guidance of policy should not mean centralized direction of the work of individual scientists, though it points out that research is not wholly a matter of individual decisions. With the increasing cost of equipment and facilities, it becomes a matter of general policy to decide what new facilities can be afforded, which are likely to make the greatest contributions to science, and which to the attainment of other national objectives. From this aspect of policy-making—the provision broadly for the advancement of science and its practical applications—arises the debate as to how far Britain should engage in space research or nuclear physics research, and the scale of any such effort relative to that, for example, in biochemistry or medical research.

This is the first class of policy problems which the Group distinguishes. The next comprises the contributions that scientific and technical knowledge can make to the solution of other national problems. In illustration the report quotes the ability to detect nuclear explosions, and the bearing of this ability on

decisions concerning disarmament and inspection. A third class includes the influence of national needs on science and technology. Political, military and economic needs all help to determine the emphasis given to different technological areas and even to different fields of research.

The report argues that whoever has responsibility for making decisions on these inter-related issues must have opportunity and authority to review the entire research and development effort of the nation, and to give due consideration to other issues of policy; the fundamental question before the Group was whether the proposed Department would provide the means for this, and even ensure a drastic improvement in the capacity of the Federal Government to cope with scientific and technical problems. With this in mind, the reports sets forth the alternative possibilities. It rejects as unworthy of serious consideration any proposal that all the research and development activities of the U.S. Government shall be brought together in one Department. Scientific and engineering activities that are intimately related to the operating responsibilities of an agency belong to that agency: research on military problems, for example, cannot wisely be divorced from the Department of Defence. Again, science and technological development have so much in common and benefit so much from each other, in any particular area, such as nuclear energy, that the two should remain under the same administrative direction.

These two principles appear to have been recognized in at least some of the proposals advanced in the United States. Dr. Berkner, for example, excluded research and development activities which are an integral part of the agencies in which they are at present located—for example, the Departments of Agriculture and Defence—as well as agencies which are primarily responsible by supporting research carried out by others. He would also exclude independent agencies that are already strong and command firm support, such as the Atomic Energy Commission and the National Aeronautics and Space Administration. In the United States this would leave a group of scientific and technical bureaux which Dr. Berkner believes could be moved without detriment to the departments in which they are already located, though there is no obvious title for the new single Department. The main advantage would be that, through the closer contact thus established, the scientific and research activities would be stimulated and the new Department could be given responsibility for the development of new scientific activities. A Department devoted wholly to scientific and technical activities would, he suggests, be more likely to be on the alert to identify areas that need special emphasis, and more eager to secure the facilities that are required.

The proposal was not accepted without modification by the Group. The report that Mr. D. Wolfle has compiled, while agreeing about certain exclusions, suggests that the National Aeronautics and Space Administration should be included, and possibly also the National Science Foundation. None of the proposals for a Department of Science and Technology was regarded as entirely satisfactory, though each helps to reveal deficiencies in the present administrative structure. Dr. W. R. Brode's proposal for a Department of Science, for example, while accepting the general principle that scientific activities essential to the work of an operating agency should be left in that agency, is designed to correct the tendency for authority to engage in basic research in fields that underlie an agency's operating responsibilities, thus supporting an unnecessarily wide range of scientific activities. This was the substance of some serious criticism in Britain of Admiralty research advanced in reports from the Select Committee on Estimates. Dr. Brode's Department of Science, while not removing from agencies such as Defence and Agriculture the research programmes specific to their missions, would include all major segments of science not specifically pertinent to those missions.

Even with the sound principles endorsed by this report, the real difficulties lie in deciding where to draw the line, and in finding the balance of advantages and disadvantages in any change of organization. The Group recognizes very clearly, for example, the value of having alternative means of allocating funds, particularly in fundamental research, as well as the difficult decisions which have to be taken about entering new fields. Here, however, its major emphasis is on the need to watch for new developments, and, whether or not a Department of Science and Technology is established, the report stresses the urgency of achieving greater competency for looking ahead to identify and develop those areas of research that are not yet the responsibility of one of the applied science agencies but that appear likely to produce major advances and applications in return for special support. It might well have been added that closely allied is the no less difficult problem of deciding when to discontinue such support and where the responsibility for continuance or discontinuance should lie.

There is little in all this to point to any need for basic changes in the present structure of Britain's organization for securing that due account is taken both of scientific and technical factors in the making of policy and for securing effective co-ordination of such activities and reasonable allocation of resources for such purposes. Broadly speaking, that is the purpose of the creation of a Ministry for Science, and the report provides substantial reasons for doubting whether that purpose will be better achieved by giving the Minister more executive responsibilities in fields which lie outside his present responsibilities, but not his interest, as in defence research. More is likely to be achieved by strengthening the informal links which at present exist, as between the Advisory Council for Scientific Policy and the Defence Research Advisory Committee, for example, between the several research councils themselves and between them and the universities and industry, and also by positive measures, through the Parliamentary and Scientific Committee and in other ways. In this way it may be possible to create an informed public

opinion prepared to provide and maintain the necessary support, competent at least within certain sections to criticize soundly, and one which also sufficiently recognizes the value and significance of science in Government to ensure that the question of status no longer arises.

There are, however, major considerations in this matter of determining overall scientific policy to which the American report directs attention. The limits within which existing or new means of presenting scientific or technological advice can function must also be recognized. Scientific and technological factors are only one element, even if an important one and on occasion the deciding factor in determining national policy. There are financial, economic, social and other factors to be considered, and in this the political element—and frequently the party political element—enters and cannot be excluded. For this reason, as the report points out, in the United States the centre of responsibility for policy must be close to the President, and this would be unaffected by the creation of any new Department of Science and Technology.

What matters is that for the Office of the President to possess the competence to exercise that role wisely, its staff must include men who are highly skilled in the management of scientific affairs, and who can relate scientific matters to all the other considerations that are involved in major decisions on policy. In this connexion it should be noted that the Group stresses the distinction between the position of Special Assistant to the President for Science and Technology and that of the Chairman of the Science Advisory Committee. Even though the two positions may be held by the same man, they are quite distinct. The Committee and its chairman should very properly place first emphasis in their thinking on the scientific and technological needs of the nation and the national implications of scientific and technological developments, and should be free to criticize established or proposed policy if damage to the nation's research and development programme is foreseen. In contrast, the Special Assistant is part of the Presidency, and must always subjugate specifically scientific or technological factors to the total of those involved in making a political decision—that is, a decision on policy. "Whether the chairman and the Special Assistant are the same man or different men, the President needs the best scientific and engineering advice available, and thus needs an Assistant for Science and Technology who can help him to weigh, judge, incorporate, and perhaps override that advice in establishing national policy."

The significance of this observation is obvious. In Britain, the Minister for Science provides the appropriate means for performing for the Prime Minister and in the Cabinet this latter function of the President's Special Assistant for Science and Technology, and it appears to rest with him also to see that through the Advisory Council for Scientific Policy and in other ways he is provided with the advice he needs for doing so. There appears to be no reason to doubt the adequacy of existing arrangements in Britain; but in the United States there seems to be room for improvement in the Federal Council for Science and Technology, and the Group appears to have had the personal factor more particularly in mind. So far as Britain is concerned, it would be difficult to suggest anyone better qualified than the present Minister for Science for presenting scientific and technological factors at ministerial level. Far more important at this level than scientific training is the ability to appreciate a situation as a whole, to work effectively with others and to present the scientific and technical issues clearly and relevantly. It is rather at the level of the advisory committees and councils and at the administrative level in the departments that the question of scientific training and administrative ability arises.

The American report commends for consideration the idea of having within each department an assistant secretary, or officer of similar senior rank, responsible for all research and development activities, and of using these officers as a strong co-ordinating council. Whether such a step would offer any advantage in Britain may be questioned. It would obviously tend to overlap the work of some of the existing advisory committees and councils and possibly to blur responsibilities and functions. Much more important seem to be the quality and the training of administrators in such posts, and the quality of the membership of the advisory councils and committees. This last in turn is linked to the question of status. Men of outstanding ability and experience and judgment will not be found to serve upon them unless the work is seen to be effective and their representations are treated with respect, even when in particular situations other factors may lead to their advice being over-ridden.

On the first problem, however, the question whether scientists rather than administrators as such should occupy more of the key posts in departments concerned with research and development merits more examination than the Government has been disposed to give it. Whether or not such an administrator is primarily a scientist, it is undeniable that it is increasingly impossible for him to be effective unless he has received sufficient scientific training and acquired sufficient scientific knowledge to appreciate the relevance and significance of the scientific or technical advice presented to him. Administrative ability is the primary criteria, but much more could well be done in the Civil Service to ensure that for scientists and technologists with the essential administrative flair there is full opportunity to rise to the highest posts. It does not speak well for the Government's handling of present conditions that there should be signs of such obvious discontent as the simultaneous day's leave taken by eight hundred of the scientific staff at Harwell last March, or the protests voiced at the recent annual conference of the Institution of Civil Servants.

It is true that, as a long article, "Scientists versus Administrators: an Approach towards Achieving Great Understanding", by Mr. Z. M. T. Tarkowski and Miss A. V. Turnbull in *Public Administration*

last autumn shows, the Treasury is now giving some attention to this question, and residential conferences for senior scientists and administrators on organization and management have been arranged in the Civil Service for some years in which administrators, scientists and professional staff have been mixed in about equal proportion. The promotion of such understanding, like the promotion of effective co-operation and co-ordination, is, however, largely a matter of providing appropriate conditions, and it might well be worth while for Lord Hailsham to give some attention to this aspect and to the removal of the obstacles to which attention is directed in this article, particularly perhaps to the provision of facilities for scientists to obtain administrative training and experience.

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The Royal Society

Its Origins and Founders. Edited by Sir Harold Hartley. Pp. ix+275+29 plates. (London: The Royal Society, 1960.) 35*s.*; 6 dollars.

A Brief History of the Royal Society

By Prof. E. N. da C. Andrade. Pp. iii+28+14 plates. (London: The Royal Society, 1960.) 10*s.* 6*d.*; 2 dollars.

ONE of the most striking features of the preparations for celebrating the tercentenary of the foundation of "a Colledge for the promoting of physico-mathematicall-experimentall learning" which in 1662 became the Royal Society has been the flood of articles on the history of almost every aspect of this great enterprise and of those activities which were afterwards associated with it. To the three works published during the tercentenary celebrations there will rightly be attached a special importance. To "The Beginning of the Royal Society", which alone of the three does not carry the Society's imprint, there may also be attached a certain notoriety since it unambiguously asserts what "Origins and Founders" implicitly denies, namely, that the Royal Society 'began' in Oxford. Though the adoption of an 'official' version would be wholly at variance with the spirit of the Royal Society, it is the case that three of the four histories of the Society written by its officers—Birch, Weld, and Lyons—all adopted the statement of Wallis against Spratt (the fourth) that the Society's "first ground and foundation" was in London. The authors of this "agonizing reappraisal", in claiming that Sprat "speaks with the voice of the contemporary [1665] Royal Society", do not add that Sprat himself freely admitted that the "objections and cavils" against the Society had compelled him to write of it "not altogether in the way of a plain history but sometimes of apology". The "voice" of the propagandist—whether individual or corporation at a critical moment of its existence—is scarcely likely to be an infallible guide in the establishment of historical truth. In support of their thesis the authors have assembled a great deal of evidence relating to the activities of the 'philosophers' at Oxford before 1660. To me it seems regrettable that this impressive picture of the high significance of Oxford in the growth of the idea which finally took shape in the Royal Society was coupled with the dogmatically presented claim that the Society 'began' there.

"Thoughts," wrote A. N. Whitehead, "lie dormant for ages, and then almost suddenly, as it were, mankind finds that they have embodied themselves in institutions." To me it is the great strength of Prof. D. McKie's long 'leading' article on "Origins and Foundation" that it reveals the Royal Society as the embodiment of a thought which had no beginning, but whose emergence in seventeenth-century England was the final act of a process discernible under various forms in the previous two centuries. The almost "sudden embodiment" was due to a combination of the genius and devotion of a few individuals and a particular historical situation. The latter, in Prof. McKie's judgment, was the influx into London of several influential Royalists (such as Viscount Brouncker and Sir Robert Moray) with a taste and aptitude for "natural philosophy"; the deployment of the former is the concern of the rest of this admirable volume. Of the twenty-one biographies included twelve are those of the men whose recorded decision is the only indisputable 'beginning' of the Royal Society (John Wilkins, Jonathan Goddard, William Petty, Christopher Wren, Laurence Rooke, Robert Boyle, Viscount Brouncker, Paul Neile, William Ball, Abraham Hill, Robert Moray, and Alexander Bruce). The remainder are of men who, though absent from that decisive meeting, were from a very early date associated in the most intimate manner with the Society's well-being: John Wallis, though an infrequent attender at meetings, of international renown and a scholar of the highest calibre and integrity; Thomas Willis and William Croone, pioneers of experimental medicine—the latter also being "named for Register"; Robert Hooke and Henry Oldenburg, without whose complementary talents and devotion the "first fine frenzy" might have evaporated; and John Evelyn, by whose suggestion the Society was granted its Royal title. The two remaining—Kenelm Digby and Elias Ashmole—were indeed less-intimately associated with the Society, but their importance for the understanding of the climate of opinion of the times fully justifies their inclusion. There is no space even to name the authors of these biographies, let alone to touch on their several merits. For the sustained enthusiasm which must have been brought to the task—evidently far greater than usually falls to the editor of such a symposium—the Republic of Letters will owe a permanent debt to Sir Harold Hartley: it will be best discharged by following the advice of the printer of Copernicus—*eme, lege, fruere*.

Prof. Andrade's booklet falls into a somewhat different category. His aim is to present a portrait of the Royal Society as a living organism active in the advancement and dissemination of natural knowledge; independent of State control, but ready to enlist State aid for projects likely to promote those ends. Since the life of an organism is determined by its mode of growth, Prof. Andrade has drawn freely on his great knowledge of the commanding figures of the past; but since no chronological sequence is maintained the title might be thought somewhat misleading. This however is a small matter; if the aim is here correctly described, the achievement could scarcely have been bettered.

William P. D. Wightman

GENETICS FOR BEGINNERS

An Introduction to Genetics

By Dr. C. M. M. Begg. (Biological Science Texts.) Pp. xi+292. (London: The English Universities Press, Ltd., 1959.) 35*s*. net.

THIS is a valuable addition to introductory text-books on genetics, suitable for undergraduates and for the reader with some knowledge of biology. It covers a wide range, from formal genetics, population genetics and evolutionary theory to the biochemistry of gene action. It is attractively produced, easy to read and lavishly illustrated with large, clear diagrams which form an integral part of the text.

It is a measure of the rapidity of growth in genetics that the chemical structure of nucleic acids in the chromosomes can now be discussed in an elementary text-book of genetics, and Dr. Begg is to be congratulated on discussing this and other questions which are not yet fully understood, and making it clear to the reader why they are not understood. This is always exciting and provoking in an introductory book. It is therefore surprising that he should have avoided a fuller discussion of the structure of the genetic unit and the nature of mutation and left the reader high and dry over the interrelationship of deoxyribonucleic acid and ribonucleic acid. It is nowhere made clear that ribonucleic acid is found both in the nucleus and cytoplasm nor that proteins always occur with the deoxyribonucleic acid in the chromosomes. This makes it difficult for the reader to effect a synthesis between the first and last chapters.

There are several misprints which may well lead the beginner astray, for example an extra plus sign has appeared in Fig. 97, and it is to be hoped that the problems posed in the references in the last chapter are not an introduction to the stated problems, with solutions, which follow.

It is a pity that small points of editing should have been allowed to mar an otherwise useful book, a book in which a great deal of effort has been made to use genetic examples from man and to emphasize that genetics is of interest to us all.

WILMA GEORGE

IDENTIFICATION OF BLOOD-GROUP ANTIGENS

The Anti-Globulin (Coombs) Test in Laboratory Practice

By Dr. I. Dunsford and Jean Grant. Pp. xiii+120. (Edinburgh and London: Oliver and Boyd, Ltd., 1959.) 12*s*. 6*d*. net.

THE antiglobulin test of Coombs introduced in 1945 was, in fact, a rediscovery, for so long ago as 1908 its principles were worked out by C. Moreschi, an Italian. Dr. Coombs was unaware of this when he introduced his test, but later he duly acknowledged Moreschi's work. The test, as devised by Coombs, Mourant and Race in 1945, is now very extensively used both in routine investigations and in research work.

Many important and substantial advances in blood group serology, that is, the identification of blood-group antigens in the cells, and isoantibodies in the serum (or plasma), would not have been possible without the Coombs antiglobulin test. In selecting blood for transfusion the Coombs test is quite the most reliable compatibility test thus far devised. This test has also proved to be extremely useful as a research instrument and, in particular, has proved invaluable in affording us a better understanding of hæmolytic anæmias. This book, written by two experienced workers, reflects the experience generally not only of the Oxford and Sheffield regional transfusion laboratories but, also, that of other transfusion laboratories. It gives an informative account of the Coombs test, the preparation of antiglobulin sera, the applications of the test, and there is a comprehensive list of references for those who wish to read further on the subject. Certainly this book will be very useful to every pathologist and technician who operates a blood bank.

A suitable account of antigens and antibodies is given, their detection by the Coombs test, and the serological basis of this test. The production, processing, and standardization of anti-human-globulin sera, the behaviour of such sera in tests, the technique and applications of the Coombs test, and the various snags which may arise, are all competently dealt with. The authors point out that antiglobulin sera prepared by heating or by dilution may be adversely affected in that they may be suitable only for detection of γ-globulin coatings. The application of Coombs's test in fields other than blood-group serology, for example in bacteriology, forensic medicine, hæmolytic disease in animals, etc., are briefly but adequately dealt with. This is a useful little book, being compact and concise, and it is to be hoped it will be kept so in future editions which will surely be required.

R. DRUMMOND

EVOLUTION OF MODERN EDUCATION

Studies in the History of Education 1780–1870

By Brian Simon. Pp. 375+24 plates. (London: Lawrence and Wishart, 1960.) 37*s*. 6*d*. net.

THE history of education often lends itself well to a Marxist analysis, and in the case of Mr. Simon's book it has undoubtedly helped to throw fresh light on British social developments in the nineteenth century. What makes this approach exasperating as well as rewarding is its tendency to straight-jacket the material, thereby first illuminating it and then obscuring it—the former by catching it in a sharp focus, the latter by distorting it to fit into a partial pattern of vision.

During 1780–1870 there was indisputably a vast social change, in relationship to which educational principle and practice stood as both cause and effect. Political and economic power shifted from the land-owning aristocracy to the Whig magnates and then, in step with technological invention, to an increasingly prosperous middle-class. This was reflected first in the attack of the Philosophical Radicals on the former establishment in Church and State up to 1832, and secondly in the building up of social institutions in favour of the liberal interest after that date and against the extension of full human rights as yet to the rest of the populace. A trifle tedious in connexion with the first of these phenomena, Mr. Simon's writing seems to catch fire as in Chapters 4 and 5 he describes

the response educationally of the still unemancipated section of the nation. Particularly good are his portrait of William Lovett as an educationist and his definition of the links between the educational and more broadly political aspirations of the Chartists.

The following passage on page 223 reveals both the strength and the weakness of the author's arguments: he quotes from the *Northern Star* (1848) a rebuke by the workers to those who in their eyes had betrayed them:

"In our day, the Educationists are still what they were in Cobbett's time—the pretended friends, but the real enemies of the people . . . Ah gentlemen, we see through your craft . . . You would educate us, not, as you sometimes pretend, to fit us for the exercise of political rights, but to make us indifferent to those rights. And you call yourselves 'Philosophical Radicals'."

"This uncompromising statement," comments Mr. Simon, "epitomises the reaction of the most advanced section of the working class to the educational tactics of the bourgeoisie." The author's strength lies in his belief in and capacity for using a partial point of view in order to invest a series of historical events with meaning: his weakness lies in a too doctrinaire use of the viewpoint. Such a phrase as 'the educational tactics of the bourgeoisie' implies the ascription of far too conscious and unitary motives to what was a non-homogeneous and changing stratum of society. It merely serves to conceal the more difficult truth that, as Homyakov expressed it, "Only that is conservative which moves forward, and only that is progressive which does not break with the past". Mr. Simon's carefully documented book with its telling illustrations should help perceptive students of English nineteenth-century educational history to assimilate that paradox. James L. Henderson

NUCLEAR MAGNETIC RESONANCE

High-resolution Nuclear Magnetic Resonance

By J. A. Pople, W. G. Schneider and H. J. Bernstein. (McGraw-Hill Series in Advanced Chemistry.) Pp. xii+501. (London: McGraw-Hill Publishing Company, Ltd., 1959.) 104*s*. 6*d*.

At some stage in the development of almost every specialized physical technique of chemistry there has appeared a text-book which has ultimately become recognized as a standard work on the subject. Such books play a very important part in the practice of chemistry because they invariably provide a convenient source from which the underlying principles of the subject to which they pertain can be mastered. It is therefore most fortunate that the technique of high-resolution nuclear magnetic resonance, which is so obviously destined to be of major importance in chemistry, has acquired a standard text so early in its history, for certainly "High-resolution Nuclear Magnetic Resonance" will admirably fill this role.

This book is the result of a fruitful collaboration of a theoretician and practical spectroscopists, and as such provides a well-balanced account of basic theory, experimental technique, and practical applications. The first part of the book, entitled "Principles", deals with theory and its relation to experiment, and includes brief, but sufficient, descriptions of actual spectrometers. An elementary knowledge of quantum mechanics is necessarily assumed and on this basis the principles of the subject are developed concisely and with considerable clarity. The only serious criticism of Part 1 concerns the generalization made in section 4–8 to the effect that the preferred method of standardizing spectra uses an external reference. Even in 1958 it was more or less recognized that suitable internal references offer considerable advantages.

Part 2 deals with the complete range of applications of high-resolution nuclear magnetic resonance to chemical problems. As the book contains comparatively few references after the first six months of 1958 the real power of proton spectroscopy is not fully indicated. Indeed, it might be argued that the two parts of the book should have been published as separate volumes on the grounds that the contents of Part 2 cannot possibly remain representative of the subject for more than one or two years, and that many private purchasers might have been glad of obtaining Part 1 at a reduced price. Nevertheless, the later chapters of the book contain much which will be of considerable interest and help to both the expert and uninitiated reader.

Such criticisms as have been made above are of minor significance compared with the stimulus which this book is already giving to a rapidly expanding subject. Certainly every worker in the field of nuclear magnetic resonance will need to know and use this book, and those in other disciplines will consult it on numerous occasions. Finally, the authors and publishers are to be congratulated on the uniformity and excellence of the presentation.

L. M. Jackman

PRINCIPLES AND PRACTICE OF TELEVISION ENGINEERING

Television Engineering

Principles and Practice. By S. W. Amos and D. C. Birkinshaw. (B.B.C. Engineering Training Manuals.) Vol. 1: Fundamentals, Camera Tubes, Television Optics, Electron Optics (written in collaboration with J. L. Bliss). Pp. 302 (1953). 30*s*. net. Vol. 2: Video-Frequency Amplification. Pp. 270 + 5 plates (1956). 35*s*. net. Vol. 3: Wave-form Generation. Pp. 226 (1957). 30*s*. net. Vol. 4: General Circuit Techniques. Pp. 268 (1958). 35*s*. net. (London: Iliffe and Sons, Ltd.; New York: Philosophical Library.)

These four volumes form a comprehensive guide to the principles and practice of television engineering produced by authors who have actively participated in the development of the B.B.C. television services for many years past. The series has been produced from text-books prepared primarily for instruction of the Corporation's own staff, and they are now made available for a wider technical public.

The first volume deals with the fundamental principles of converting a visual scene into electrical signals for transmission to a distant receiver. Considerable space is given to a description of the various types of television camera tubes, and a discussion of the principles of the transmission of light by lenses and mirrors, and of the analogous problems encountered in electron optics. Volume 2 is devoted to video-frequency amplification at both high and

low frequencies, with some reference to the sources and nature of noise associated with camera-head amplifiers. In this case, the text is rather more mathematical than in the first volume; but a wide variety of circuits is described, including the use of feedback. Numerical examples are given to illustrate the application to practical equipment.

A somewhat similar presentation is adopted in the third volume, which covers the subject of wave-form generation for television transmission. Separate parts of this book are devoted to the production of waves of sine, rectangular, sawtooth and parabolic form, with details of the various types of valves and circuits suitable for these. The multi-vibrator, in one or other of its different forms, naturally plays a large part in this technique as applied to the reception and analysis of television signals.

The fourth and last volume of this comprehensive work deals with a wide range of circuit techniques, many of which are applicable to the general field of electronics as well as to television engineering. Among the techniques treated are counter circuits, frequency dividers, direct current clamping and restoration, and amplifiers, delay lines and equalizers. Some of these subjects require a mathematical treatment but, in order to facilitate the presentation of the text, many of the mathematical derivations are given in appendixes to the individual chapters.

Altogether these four volumes form a most valuable aid to those engaged in the science and application of the generation and use of television signals, and allied fields of electronics. The texts are very clear throughout and well illustrated by graphs and schematic circuit diagrams. The production of the volumes is of a high standard and is a credit to the publishers as well as to the technical staff of the British Broadcasting Corporation. The work can be confidently recommended as a guide to circuit designers, as a training manual to lecturers and as a general work of reference to the radio and electronics engineer.

R. L. SMITH-ROSE

UNIFICATION OF ANALYTICAL APPROACHES TO ENGINEERING PROBLEMS

Engineering Systems Analysis

By Prof. Robert L. Sutherland. (Addison-Wesley Series in Instrumentation and Control.) Pp. xii+223. (Reading, Mass.: Addison-Wesley Publishing Company, Inc.; London: Academic Books, Ltd., 1958.) 60*s*.

THE unification of the analytical approach to engineering problems is an ideal which is sometimes attempted in advanced texts on certain branches of engineering science. It is not, however, very commonly attempted in text-books for undergraduates. Clearly there is a major difficulty in limiting the scope of such a work and in selecting the material to be included. It is, therefore, particularly interesting to find an undergraduate text-book under the title "Engineering Systems Analysis".

The book opens with a study of basic electro-mechanical analogies and then proceeds to a consideration of mechanical vibrating systems. This is followed by a chapter on oscillations in electrical networks. A section dealing more generally with dynamical systems develops the method of treatment for mixed systems with electrical and mechanical components and explains the use of dimensionless ratios. Dimensional analysis is then treated in considerable detail and its application is illustrated by examples in the field of hydro-dynamics. Thereafter follows a section on the principles of feedback and control and the book concludes with a chapter on analogue and digital computing machines.

With such a wide coverage it might be thought that the treatment would be too superficial to be of real value. In fact the author has succeeded in dealing with a selection of fundamental topics in quite sufficient depth and at the same time in producing a coherent whole. This has in large measure been achieved by the judicious selection of his illustrations.

It would appear from the author's preface that this text-book will meet the needs of certain courses which are being developed in universities in the United States. It is a little difficult to see precisely where this book would fit into the structure of most British undergraduate courses in engineering. None the less, the unification of treatment which is achieved is something of intrinsic value which merits the attention of undergraduates, and the book can certainly be commended as basic reading for both mechanical and electrical engineers. The standard of mathematics involved should be well within the competence of the British undergraduate in his second year.

J. GREIG

RUBBER TECHNOLOGY

Introduction to Rubber Technology

Edited by Maurice Morton. Pp. v+547. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1959.) 80*s*. net.

AN "Introduction to Rubber Technology", edited by Prof. M. Morton, of the Institute of Rubber Research of Akron University, Akron, has first to be compared with the standard work on "Synthetic Rubber" edited by Morton's predecessor, G. S. Whitby. "Synthetic Rubber" is primarily concerned with the preparation and uses of the general purpose styrene-butadiene rubber, with relatively brief mention of chloroprene, nitrile, and butyl rubbers. "Introduction to Rubber Technology" devotes the same fraction of its space to styrene-butadiene rubber as to each of the other rubbers considered. There is no case, therefore, to regard the new book as overlapping seriously the older.

After introductory chapters on the history of rubber technology and polymer chemistry, separate chapters deal with natural rubber, butadiene-styrene, nitrile and acrylate, butyl, polychloroprene, chlorosulphonated polyethylene, polysulphide and silicone rubbers. The various types of rubber additives—plasticizers, antioxidants, and anti-ozonants, accelerators, and fillers—are also treated in separate chapters. Articles on reclaimed rubber, latex and sponge rubber, physical testing, and processing equipment round off a broad survey of modern rubber technology. The only serious omission appears to be of the new polyurethane and polyether foams.

Morton has achieved a large measure of success in welding together the efforts of twenty contributors. The technical level of the articles is uniform and makes for a very readable book. The material is up to date, and presents for the first time in book form much information of value to rubber technologists in

Britain. The quality of printing and of the illustrations is first-class.

The question does arise as to whom this book is directed. It will probably be disappointing to specialists in other fields who seek from the title an introduction to rubber technology within the framework of chemistry and physics. The policy appears deliberate of keeping the scientific matter on a 'popular' level. For example, in the chapter on polymer chemistry, one jumps within a page from atoms and valency to monomers and polymers. Again, in the chapter on vulcanization, the concept of cross-linking is dismissed with reference to the analogy of a fish net of varying size of mesh, and no concept is given of the number of cross-links to monomer units or dependence of properties on the nature of the cross-links, to take only two relevant subjects which should surely be dealt with. The popular approach is also sometimes misleading, as in the equations put forward to describe rubber oxidation.

Rather than an 'Introduction', the various chapters of this book are really self-contained reviews. As such, they include much of interest to practising rubber technologists. The readers of this book will, in general, be already qualified and could with ease have swallowed stiffer doses of the science of their subject from the eminent contributors to this volume. A chemistry and physics of rubber technology still requires to be written.

W. F. Watson

MARINE BIOLOGICAL REPORTS FROM DENMARK

Meddelelser frå Danmarks Fiskeri- og Havundersøgelser

Ny Serie, Bind 2, Nr. 23–26. Nr. 23: Investigations on the Quantitative and Qualitative Distribution of Zooplankton in the Southern Part of the Norwegian Sea. By V. K. Hansen. Nr. 24: A Quantitative Investigation of the Echinoderm Fauna of the Central North Sea. By Erik Ursin. Nr. 25: Conditioning *Ostrea edvlis* L. from the Limfjord for Reproduction out of Season. By I. A. Aboul-Ela. Nr. 26: The Oligochaete Fauna of Three Danish Brackish Water Areas. By I. Dahl. Pp. 292+5 plates. (København: C. A. Reitzels Forlag, 1960.) 47 Danske kr.

THE final part of the second volume of the new series of biological reports published by the Danish Institute for Fisheries and Marine Research contains four interesting contributions which carry on the high standards established by the earlier series. The first paper, by V. K. Hansen, "Investigations on the quantitative and qualitative distribution of zooplankton in the southern part of the Norwegian Sea", describes the results of cruises made during several seasons in the area of the off-shore herring fisheries just north of the Iceland–Faeroes–Shetland ridge. Samples were taken with the vertical Hensen net, supplemented by horizontal hauls with small high-speed samplers of the Hardy Indicator type. The catches are described and discussed in relation to movements of Arctic and Atlantic water masses, but conclusions are apparently being kept until the remainder of the plankton community has been described.

Of two shorter papers, one by I. A. Aboul-Ela, on "Conditioning *Ostrea edulis* L., from the Limfjord for reproduction out of season", shows that successful spawning can be induced by higher temperatures. The other paper, by Mrs. Ingerlise Dahl, on "The Oligochaete fauna of three Danish brackish water areas", deals with the systematics and biology of these little-studied animals, which form a surprisingly important component of the bottom fauna in the Danish Fjords.

Most of the volume is taken up by Dr. E. Ursin's comprehensive study, "A quantitative investigation of the Echinoderm fauna of the central North Sea". The author has based his work on most of the Danish material collected since 1922, as well as on a large number of quantitative grab-samples taken from R.V. *Dana* during 1950–55. Full tables are given showing the occurrence of the commoner species at all stations since 1932, and the distribution of both common and rare forms is described in some detail. Reference is made to the accepted bottom fauna communities, but in the main the distributions are considered from the zoogeographical point of view in comparison with other localities, notably Iceland and the Adriatic. Apparently the echinoderms of the southern North Sea have decidedly southern affinities, and the total numbers per unit bottom area are considerably greater than in the northern part. However, the author regards the latter area as the rich area for echinoderms because a larger number of (rarer) species occur there. Many of the species found only in the northern (or north-western) part of the North Sea are distributed to the north of the area (for example, *Leptasterias mülleri*, *Strongylocentrotus droebachiensis*), and would thus be expected to be limited to the northern region because of the generally cooler conditions there during the summer months and the somewhat deeper water which allows the establishment of a thermocline. Other, much fewer, species of southern distribution outside the North Sea (for example, *Antedon bifida*, *Palmipes membranaceus*) are also limited to the same area as the cold-water forms, possibly, it would be expected, because they can better survive the colder months of the year there than farther to the south and east where there are greater extremes of climate. However, the author, after considering such hypotheses, tends to reject most temperature correlations, and instead compares the distributions of the rarer species with the distribution of water masses characterized by the plankton community represented by the chaetognath *Sagitta elegans*.

As one reads the accounts of individual species one gains the impression that the author considers that the presence of "*Sagitta elegans* water" is the predominant environmental factor governing the distribution of echinoderms. Even if the experiments on biological factors in such water are considered conclusive, it is difficult to believe that they can have much influence on non-pelagic larvæ or on the adults themselves. Many inconsistencies in distribution in the North Sea would be more intelligible if other environmental factors were considered. Not only temperature but also depth of water, type of deposit and continuity of faunas must be of more direct importance to most species of echinoderms than hypothetical factors associated with water masses. In conclusion, the possibly subjective interpretation must not be allowed to detract from the general importance of this paper, which will form the basis for much future discussion, and zoogeographers will be grateful to Dr. Ursin for his careful compilation of the distributional records.

A. J. Southward

WESTFIELD COLLEGE (UNIVERSITY OF LONDON)

NEW FACULTY OF SCIENCE

SINCE its return to London after the break of the war years, Westfield College (University of London) has been a College in the Faculty of Arts only, except for mathematicians, who have taken either the B.Sc. (Special) or B.A. degree. Previously, the College had a small, but flourishing, Department of Botany, but this was relinquished in 1950 as a result of the difficulties encountered in running one science department in isolation.

In November 1955 the College Council decided to press for the re-introduction of science, but on a wider basis, and the advice of Sir Lawrence Bragg and other eminent scientists was sought and most kindly given. The reasons for the decision probably need no advocacy, but are summed up as follows: "We are convinced that the College would gain immeasurably by the re-introduction of Science: past experience has shown that the contact between students of Science and students of the Arts, living and working side by side, is of the greatest benefit to both, in ways difficult to analyse but none the less real". The original plan was to establish a Faculty of Science "comprising Special Honours Departments of Chemistry, Physics, Botany and Mathematics (already in being), with in addition a two-year Zoology course" (afterwards included as a special honours department) and that these departments should "at the same time cover the requirements of the B.Sc. General Honours Degree". The shortage of science teachers in the schools was kept in view, and it was thought that "there might well be a temporary paucity of properly qualified candidates for Special Honours courses (but) . . . that to have the stimulus of Special Honours Departments is the right policy in the long view". A Faculty of approximately two hundred students was envisaged, though this was increased later in the light of advice from the University. The College possessed its old botany laboratories, equipment and materials, which had been retained, and the botany library, where periodicals and series had been kept up to date. It also had a site for extensions.

In June 1956 a small body of experts appointed by the University went to examine the building plans and confer about staffing and accommodation. Their opinion was that the site proposed was too restricted, and an alternative site allowing for more research facilities as well as possible expansion had to be found. It was also stated by the University when examining the estimates that the original plan was too modest and that if the plan were to be given ultimate sanction the College should think in terms of a building costing about half a million pounds, and also that the building must be ready to accept students in October 1961. The building plan was modified more than once to accord with London County Council requirements and finally it became evident that to obtain a site of sufficient dimensions one of the College residences would have to be demolished.

In all these negotiations the College was helped by the criticism and advice of the inspectors appointed by the University, some of whom afterwards became 'science advisers' to the College and to whom it is deeply indebted, notably Prof. H. T. Flint (then Hildred Carlile professor of physics at Bedford College), Prof. W. S. Bullough, professor of zoology at Birkbeck College, and the late Prof. W. Wardlaw, professor of chemistry at Birkbeck College. After his death in December 1958 his successor, Prof. W. G. Overend, consented to act as adviser in chemistry. In botany no special adviser was sought, since there were some past members of the College who had specialized knowledge. The architects responsible for the laboratories are Messrs. Verner Rees, Laurence and Mitchell. The actual demolition of the property to clear the site began in May 1959, and the official laying of the foundation stone of the new building was performed by Sir John Cockcroft in May 1960. Meanwhile, the intellectual foundations have been laid in the present academic year by the appointment of the first professors and by the recognition by the University of the College as a School in the Faculty of Science.

The appointments are as follows:

PHYSICS. *Prof. E. H. Bellamy.* Dr. Bellamy was educated at Quarry Bank High School, Liverpool, and proceeded to King's College, Cambridge, as a State Scholar in 1941. He obtained a first class in the Natural Sciences Tripos in 1943 and then worked for three years as a scientist in the Road Research Laboratory, Department of Scientific and Industrial Research and at British Insulated Callender's Cables, Ltd. Returning to Cambridge after the War, Dr. Bellamy completed a double-first in the Natural Sciences Tripos in 1948 and thereafter did research work in the Cavendish Laboratory for the Ph.D. degree, which he obtained in 1951. During this latter period Dr. Bellamy made the first measurements in Europe of nuclear spins and magnetic moments using atomic beam techniques. He was appointed to a lectureship at the University of Glasgow in 1951 and rendered valuable help in the development of experimental techniques for utilizing the beam of the 300-MeV. synchrotron which operates there. During the past five years, Dr. Bellamy has made many contributions to our knowledge of photoproduction of π-mesons and has very successfully supervised young research students who have collaborated with him in these and other experimental studies. He is at present on short leave of absence to carry out an experiment on the 1,000-MeV. synchrotron at Frascati. Dr. Bellamy has also taken a full share in departmental teaching and social activities at Glasgow, and has been very popular with undergraduate students.

CHEMISTRY. *Prof. W. Klyne.* Dr. Klyne was educated at Highgate School and New College, Oxford, where he read chemistry. Since then he has worked in

Fig. 1. Photograph of artist's drawing of the new Faculty of Science Building, Westfield College. (Artist: William Suddaby. Photographer: Sydney W. Newbery)

the Department of Medical Chemistry in the University of Edinburgh and in the Department of Medical Pathology at the Postgraduate Medical School of London, where he has been reader in biochemistry since 1952. Prof. Klyne has written "Practical Chemistry for Medical Students", which has been widely used. He is an expert and master of stereochemistry, particularly in regard to the biological steroids and the relation of optical rotation and rotary dispersion to chemical structure. His approach to the subject ranges from biochemistry and organic chemistry to the theoretical aspects of the shape of molecules. His interest in scientific method and his past experience should ensure success for this new department of chemistry.

Botany. *Prof. G. E. Fogg.* Dr. Fogg needs no introduction to most biologists for he is well known as a secretary of the Society for Experimental Biology, as one of the co-editors of the Penguin "New Biology" series and as the author of the book "Metabolism of Algae". Dr. Fogg took his degree in botany at Queen Mary College, London, and went to Cambridge to do his postgraduate research. Since 1945 he has been an invaluable and distinguished member, first as lecturer and now as reader, of the Botany Department in University College, London. Dr. Fogg's research work on the metabolism of algae, involving as it does the growing of algae in pure culture and the use of modern techniques, has been distinguished for its skilful, experimental approach. More recently, he has extended this work to the study of the physiology of phytoplankton in fresh water and the sea. Around this new and expanding field of research, Dr. Fogg will be able to build a sound teaching department at Westfield College.

Zoology. *Prof. J. E. Webb.* Dr. Webb returns to Great Britain after a period of eleven years as head of the Department of Zoology in the University College of Ibadan, Nigeria. After graduating from Birkbeck College, London, he held for a short time a post as research entomologist before taking up a lectureship at Aberdeen. His many publications during this period include important contributions on the morphology and ventilation mechanisms of insect tracheae. The creation and development of a flourishing department at Ibadan and of an attractive and well-stocked zoological garden are a tribute to his energy and powers as an organizer. Under his leadership and guidance the Department has acquired a well-deserved reputation for good teaching and research. Many of his students have graduated with honours degrees under the scheme of special relationship with the University of London and have taken up teaching and research posts in Nigeria; two of them are on the staff of the Department. Prof. Webb, besides playing an active part in the affairs of the University College, as dean of the Faculty of Science and member of the College Council, has established himself as an authority on the systematics of the cephalochordates, and his most recently published work, in the *Philosophical Transactions of the Royal*

Society, includes studies on the geomorphology and ecology of the lagoon systems of Lagos and the adjacent coastline.

All these professors will hold office from October 1960, in order to be responsible for final preparations and for appointment of staff before the first intake of students in October 1961. They will select their first students in November 1960, and the schools have already been circularized.

MATHEMATICS. *Prof. E. H. Sondheimer.* The Mathematics Department is in a different position, since the College never ceased to accept students for the B.Sc. special degree. The Department (about fifty students) is at present directed by a reader, but it had long been decided to apply for a chair in due course. The introduction of the Faculty of Science made it desirable that the change should take place now and the first professor of mathematics is Dr. E. H. Sondheimer. He is, at present, University reader in applied mathematics in Queen Mary College, London, a post which he has held since 1954. Prior to this, he held a lectureship in mathematics in the Imperial College of Science and Technology, to which he was appointed in 1951. This was, in fact, his first teaching post. Dr. Sondheimer has had a varied and interesting career; after taking the Natural Science Tripos at Cambridge in 1943, he joined a small team carrying out war work on experimental studies of the properties of transformer sheet steels. At the end of the War he began research work in the electron theory of metals at Cambridge, to which subject he has made several important contributions. He was awarded a Smith Prize in 1947 and in 1948 elected to a research fellowship at Trinity College which he took up in 1950. In the intervening years he worked first under Prof. N. F. Mott at Bristol and later was in the United States, working with Prof. Slater at the Massachusetts Institute of Technology. Dr. Sondheimer is best known for his work on the anomalous skin effect in metals, which was done in collaboration with G. E. H. Reuter. Later extension of this work paved the way for Pippard's pioneering work on determination of the Fermi surface in metals. Dr. Sondheimer's other important contributions include the first successful solution of the Bloch integral equation, the application of variational methods to conduction phenomena in polar semi-conductors, and a general proof of the validity of Kelvin relations occurring in the theory of phonon drag.

CONTROL OF PRE-NATAL GROWTH IN MAMMALS

By DR. ANNE McLAREN

Agricultural Research Council Unit of Animal Genetics, Institute of Animal Genetics, King's Buildings, Edinburgh 9

AND

DR. DONALD MICHIE

Department of Surgical Science, University New Buildings, Edinburgh 8

TO some extent every mammal decides for itself what size it will attain, in the sense that its own genetic constitution sets limits to its growth. However much we feed a Shetland foal it will not grow as large as a Shire horse. But there is evidence that these intrinsic factors exert their main effects in post-natal life, and that pre-natal growth is largely controlled by the intra-uterine environment.

An early indication of this was Walton and Hammond's[1] remarkable demonstration that the new-born foals of Shire mares crossed to Shetland stallions were almost as large as pure Shire new-borns, while the offspring of the reciprocal cross were as small as new-born Shetlands. The presumption was that the uterine environment was responsible, although the possibility of a cytoplasmic effect was not excluded.

Subsequent work with man[2], cows[3], rabbits[4], sheep[5] and mice[6] has confirmed the existence of large maternal effects upon weight at birth, and in the last three cases it has been shown by means of egg transfer that the effects are indeed mainly uterine.

Equally clear evidence of the importance of factors extrinsic to the embryo is the well-known effect of litter-size on weight at birth in polytocous species. According to Healy, McLaren and Michie[7], when the range of litter sizes in mice is artificially increased, differences of more than two-fold in the mean weights of full-term fœtuses can be obtained. The classical explanation of such effects is that there is a limited pool of nutriment in the maternal blood for which the fœtuses compete[8]. As each draws its ration, so much the less remains for the others.

In this article we review evidence suggesting that this explanation is incomplete, and that most effects of litter size on fœtal growth can be given a more plausible explanation in the elementary principles of hydrodynamics to which the flow of blood to the placenta is subject.

In the case of species with a bicornuate uterus, the theory of a limited pool of nutriment predicts that the growth of a fœtus in one uterine horn will be just as much retarded by the presence of litter-mates in the other horn as in the same horn. But Eckstein, McKeown and Record's[9] study of guinea pig pregnancies showed this not to be the case. They found that the number of embryos in the same horn was more important than the number in the opposite horn in determining both fœtal weight and the weight of the placenta, on which fœtal growth in large measure depends. The picture which emerges from their results can be summarized as in Fig. 1.

With regard to the possible mechanism underlying this scheme, Eckstein *et al.* pointed out that since resistance to blood flow through the placenta is low, the pressure at which blood is supplied to each placenta may be inversely related to the number of placentæ supplied by the uterine artery on the same side. It can be supposed that the supply of blood, and of its dissolved nutrients, conditions the rate of

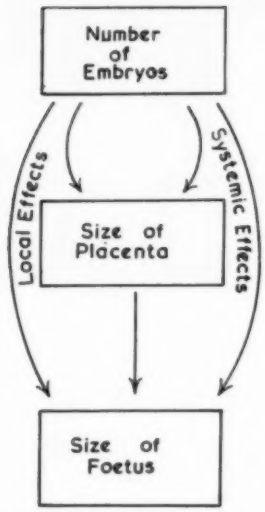

Fig. 1. Scheme showing the pathways by which the number of implanted embryos can affect fœtal growth, as suggested by the work of Eckstein, McKeown and Record (ref. 9). Reproduced by permission of the Royal Society

fœtal growth, both directly, and also indirectly by determining in the earlier part of pregnancy the size attained by the placenta. The local effect of the number of embryos is in this way neatly accounted for, and the authors pointed out that in order to accommodate the residual non-local, or systemic, effect it is only necessary to postulate that changes of blood pressure on one side are to some degree transmitted to the contralateral uterine artery.

In the course of an experiment on the induction of superpregnancy in mice[10], large numbers of full-term fœtuses were weighed, with results[7,11,12] which support this attractive hypothesis. The existence of substantial local effects on fœtal growth was confirmed. The effects, both local and systemic, of embryos surviving into late pregnancy were paralleled by effects of almost equal magnitude exerted by embryos dying in mid-pregnancy. These effects can scarcely be accommodated by the nutritional pool theory, since the nutritional demands of such embryos must be extremely small. But on the hæmodynamic theory the result is reasonable, for by surviving long enough to form a placenta, such an embryo will limit the size attained by the placentæ of other embryos and hence will limit their subsequent growth.

In addition, an extreme test of the theory of competition for a limited nutritional pool was afforded by the induction of superpregnancy in sexually immature females. These animals, as might be expected, show marked signs of nutritional stress[13], yet their full-term fœtuses were not significantly lighter than those of a parallel series of superpregnant adults (*see* Fig. 2).

It seems sufficiently clear that the limited pool theory is inadequate. At the same time, other features of the observations conformed rather well with expectation on the basis of the hæmodynamic theory of Eckstein *et al.*

The supply of blood to each uterine horn of the mouse is derived from offshoots of a main loop artery fed from the abdominal aorta at the top and from the iliac artery at the bottom. In such a system the pressure of blood must be highest in the top and bottom offshoots, decreasing in the offshoots towards the middle. We might consequently expect the larger fœtuses to be at either end of the horn and the smaller ones to be in the middle. This is precisely what has been found[11], with a particular exception which, however, helps to establish the rule. The exception is the topmost fœtus of all, which, especially in crowded horns, is usually supplied by one limb of a bifurcated offshoot of which the other limb supplies the ovary. This fœtus, especially in crowded horns, is on average smaller than its neighbour[7].

The factors controlling fœtal growth which have so far been discussed are summarized diagrammatically in Fig. 3, which is based on the suggested relationship between fœtal growth and uterine blood flow.

Another case of bifurcation relates to the phenomenon of placental fusion[12], where the fœtuses involved also have to divide between them the supply from a single offshoot. Here again, retardation of growth is found. On the other hand, in fused pairs of which one member has died in mid-pregnancy, the survivor presumably enjoys the full output of one arterial offshoot, a circumstance which tallies with the finding that such fœtuses are not significantly retarded relative to their non-fused hornmates.

In general, the hæmodynamic theory seems to account well enough for variations in fœtal growth other than those due to genetic differences between pregnant females.

There is, however, an important exception to which Hammond's concept of limitation of nutrients has a clear application. In some species the young are born at a relatively advanced stage of growth and development, so that their terminal demands upon maternal nutrition are large. One such species is the sheep, in which Wallace[14] has shown that the weight of the young at term, particularly in twin pregnancies, can be drastically altered by varying the plane of maternal

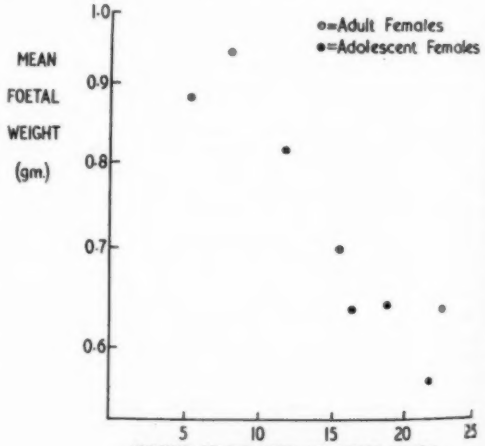

Fig. 2. Mean weights (scaled logarithmically) of 18-day live mouse fœtuses plotted against the mean number of implants surviving beyond day 10. Each point is based on 5–7 superpregnant females. In contrast to the large effect of litter size, effects of maternal age are small or non-existent

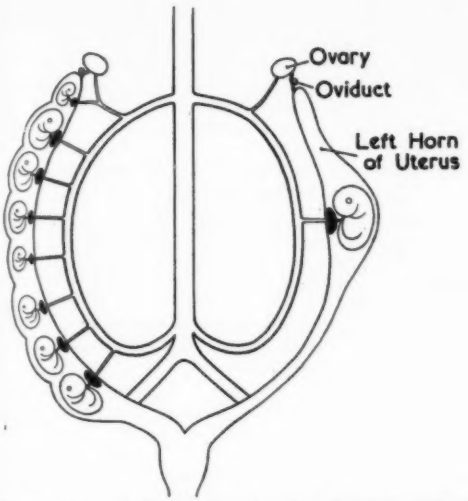

Fig. 3. Diagrammatic summary of various factors controlling fœtal growth. The one-to-one relation between fœtuses and vascular offshoots is an over-simplification. Note retardation of growth caused by (*a*) more fœtuses in the horn, (*b*) position of top fœtus in the horn, and (*c*) increasing distance from the ends of the horn. The correlation between placental and fœtal sizes is also indicated

nutrition during the last 6 of the 21 weeks of pregnancy. No effect could be obtained at earlier stages.

Such results do not, of course, exclude the simultaneous participation of the hæmodynamic mechanisms discussed earlier. That these mechanisms may have application to the human species is suggested by the finding that mothers who smoke give birth to babies weighing, on average, 6 oz. (170 gm.) less than the babies of non-smokers[15]. Since smoking causes peripheral vasoconstriction of about half an hour's duration, it is quite possible that repetition many times a day might restrict the placental circulation sufficiently to affect the growth of the fœtus. It remains to be seen whether such differences in weight at birth as that between the Shire–Shetlands and Shetland–Shires of Hammond and Walton can be accounted for in terms of vascular architecture and the mechanics of fluid flow.

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NATURE OF THE HÆM-BINDING GROUPS IN NATIVE AND DENATURED HÆMOGLOBIN AND MYOGLOBIN

By Dr. JOAN KEILIN
School of Veterinary Medicine, University of Cambridge

HÆMOGLOBIN is very widely but somewhat irregularly distributed in Nature[1,2]. It is found in all vertebrates with the exception of certain bloodless fish living in the antarctic seas, and in many species of different phyla of invertebrates. Its presence has also been demonstrated in a few ciliates among the Protozoa, in certain strains of yeasts and moulds and in the root nodules of leguminous plants.

Although the hæmoglobins, irrespective of their origin, have the same fundamental property of reversible oxygenation and have the same hæm nucleus as their prosthetic group, they differ greatly in their biological, chemical and physical properties[2] such as their affinity for oxygen and its dependence on *p*H, their affinity for carbon monoxide, molecular weight, solubility, ease of crystallization and crystalline form, the amino-acid composition of the protein, the isoelectric point, electrophoretic behaviour, ease of denaturation and immunological properties. Different hæmoglobins have been found within the same species of animals and even in the same individual, and extensive investigations are now being carried out on different genetically controlled hæmoglobins in man, cattle, sheep and other animals[3–5].

One may therefore ask what is the structural peculiarity which must be common to the proteins of all the hæmoglobins, irrespective of their origin and the great diversity of their properties, that imparts to the identical hæm nucleus (protohæm) the remarkable and not easily imitable property of combining reversibly with molecular oxygen, while at the same time protecting its divalent iron from oxidation. This is one of the most important problems which faces all those engaged in the study of hæmoglobin. That this fundamental property depends on the integrity of the protein moiety is clearly shown by the fact that the slightest disturbance in its structure is immediately reflected in the ability of its divalent iron to combine reversibly with molecular oxygen, while denaturation of the protein results in the complete loss of the property of oxygenation. In this respect hæmoglobin is, if anything, more fragile than a number of enzymes.

Although in hæmoglobin, as in other hæmoproteins, it is the entire protein portion of the molecule that influences the properties of the hæm, the first and most convenient approach to the study of this problem is to determine the nature of the groups which link the protein to the hæm iron; these will be referred to as the hæm-binding groups of the protein.

The main object of this article is to evaluate the different views which have recently been put forward as to the nature of the hæm-binding groups and to introduce some new criteria which can be applied

to their study, not only in mammalian hæmoglobin and myoglobin but also—a point of great importance—in hæmoglobins of different origins, which differ widely in the amino-acid compositions of their proteins.

Nature of the Group linking the Hæm Iron to the Protein

In considering the nature of the hæm-binding group of globin, the following four possibilities have so far been suggested: (1) the imidazole group of histidine; (2) the carboxyl groups of aspartic and glutamic acids, (3) the sulphydryl group of cysteine; and (4) no specific group.

(1) *The imidazole group of histidine.* The most widely held view is that the iron-linked group of globin is the imidazole ring of histidine. This was at first suggested mainly because mammalian hæmoglobin is rich in this amino-acid; and later this view received strong support from an extensive study of certain physico-chemical properties of hæmoglobin which we owe primarily to Wyman[6] and to Coryell and Pauling[7]. The imidazole ring of histidine as the hæm-linking group has already been discussed on several occasions[6,8,9] and will be dealt with farther on.

(2) *Carboxyl groups.* It was recently suggested by O'Hagan[10] that the hæm iron is bound to a carboxyl group of globin, since the *p*H stability curves of methæmoglobin obtained by the oxidation of hæmoglobin, or by its reconstitution from native globin and hæmatin, show ionizing groups with pK 4·7. This value lies closer to the pK of the carboxyl groups of aspartic and glutamic acids than to that of the imidazole group of histidine (pK 5·3). This view was also supported by Haurowitz[11].

(3) *Sulphydryl group.* The possibility that the hæm iron is linked to globin through a sulphydryl group of cysteine was at first suggested by Haurowitz[12], who later abandoned this view when myoglobin was found to be free of cysteine[13]. The idea was, however, recently revived by Riggs[14,15], who found that the magnitude of the Bohr effect in mammalian hæmoglobins was proportional to the number of sulphydryl groups which could be titrated by mercuric ions and that the hæm–hæm interaction, which is responsible for the *S*-shaped oxygen dissociation curve, is abolished when the sulphydryl groups are blocked by *p*-chloromercuribenzoate. He concluded that the two physiologically important properties of hæmoglobin, the Bohr effect and the *S*-shaped dissociation curve, are both mediated by sulphydryl groups which link the hæm iron to the globin. He also suggested that much of the physico-chemical evidence presented in favour of the imidazole ring as the hæm-linking group could equally well apply to the sulphydryl group.

(4) *No specific group.* Having discarded the possibility of the sulphydryl nature of the hæm-binding group, Haurowitz and Hardin[16] suggested "that the common feature of all globins is a complementary 'anti-heme' pattern which permits them to approach very closely the flat heme structure", so that the "hemoglobin structure would be maintained by London – van der Waals forces between the molecular surfaces of the heme and the anti-heme pattern of the globin". Since this view does not refer to the protein groups specifically linked to the iron, it may be considered as complementary to each of the previous hypotheses.

For the proper evaluation of these views on the possible nature of the hæm-linked groups in hæmoglobin, it is necessary to consider the reactions of free hæm and hæmatin with different substances, and to determine the requirements for the formation with some of them of the corresponding hæmochromogen and parahæmatin compounds.

Formation of Hæmochromogens by Hæm with Nitrogenous Bases

Hæm, the prosthetic group of hæmoglobin, is a flat molecule consisting of four pyrrol nuclei joined by methene bridges to form a porphyrin ring, with a divalent iron atom occupying the centre. Of the six co-ordination valencies of the iron, four lie in one plane and link it to the nitrogen atoms of the pyrrol nuclei, whereas the remaining two valencies, the fifth and sixth, one on each side of the flat hæm molecule, link it to molecules of water. An alkaline solution of hæm shows two diffuse absorption bands at about 580 and 560 mμ. The addition of pyridine to the hæm solution at once gives a pyridine hæmochromogen compound with two strong absorption bands in the visible region; α at 557 and β at 525 mμ ($\alpha \gg \beta$). Analogous hæmochromogens can be obtained with a great variety of nitrogenous bases[17,18] such as piperidine, pyrrolidine, nicotine, various amines, imidazole, ammonia, hydrazine hydrate and others. In all hæmochromogens the hæm iron is linked by covalent bonds in positions 5 and 6 to two molecules of the base[19], which replace the molecules of water.

Cyanide, unlike other nitrogenous substances, forms two distinct compounds with hæm: monocyan- and dicyan-hæm. While the general pattern of the absorption bands of monocyan-hæm resembles that of a hæmochromogen, the pattern of the dicyan-hæm spectrum differs from it and is very similar to that of the dihydroxy-hæm compound[20]. Monocyan-hæm has a strong affinity for nitrogenous bases; for example, with imidazole it forms a hybrid imidazole–cyanide hæmochromogen in which one of the two positions around the iron (fifth or sixth) is occupied by cyanide, the other by a molecule of imidazole. Such a hybrid hæmochromogen can also be obtained by treating an ordinary hæmochromogen with cyanide, which replaces one of the two molecules of the base linked with the hæm iron[21]. In this reaction the absorption bands in the visible region of the imidazole hæmochromogen are shifted to longer wavelengths, their positions in the imidazole cyanide hybrid hæmochromogen being α, 560 and β, 530 mμ, while the height (EmM) of the α band falls from 0·28–0·32 to about 0·17.

In order to find what chemical groupings in a substance determine its ability to combine with the hæm iron by covalent bonds in positions 5 and 6 to form a hæmochromogen, I have examined a great variety of substances with regard to their reactions with hæm (unpublished work). It was found that hæm formed no hæmochromogens with alcohols, aldehydes, carbohydrates, fatty acids or phenols. Only substances containing a free nitrogenous group (as has been shown earlier) combine with the hæm iron to give the corresponding hæmochromogen compounds. Since not all nitrogenous substances were found to have this property, it is of interest to record here some of the biologically important nitrogenous substances which formed no hæmochromogens with either protohæm or other hæms

(hæmatohæm, deuterohæm, mesohæm, coprohæm and urohæm), although the experiments were carried out under a great variety of conditions. The list of nitrogenous substances which failed to give hæmochromogens included urea, guanidine, creatine, creatinine, pyrrol, indol, indolacetate, proline, purines such as adenine, guanine, hypoxanthine, xanthine, uric acid, caffeine, theophylline and theobromine, pyrimidines (cytosine and thymine), adenosine, nucleic acid and its derivatives, and flavine. It is interesting to note that of all the purines and pyrimidines tested, only the unsubstituted free bases, purine and pyrimidine, gave a hæmochromogen.

The divalent iron of hæmochromogens easily undergoes reversible oxidation to the tervalent iron of the corresponding parahæmatins, which have a diffuse absorption band at about 530 mμ with a shoulder at 558 mμ. In a similar way, the oxidation of a hybrid hæmochromogen gives the corresponding hybrid parahæmatin. The formation of this compound was discovered[22] in early work on the inhibition by cyanide of the oxidation of cysteine catalysed by pyridine hæmochromogen. It was shown by Drabkin[23] that this hybrid parahæmatin can be obtained by treating hæmatin with cyanide and pyridine in the ratio of 0·1 to 5,000.

The oxidation-reduction relationship:

hæmochromogen ⇌ parahæmatin

is analogous to those of:

hæm ⇌ hæmatin and hæmoglobin ⇌ methæmoglobin.

Carboxy-hæm and Carboxy-hæmochromogen Compounds

On treating hæm with carbon monoxide, its colour turns from reddish brown to bright red and its very diffuse absorption bands at 580 and 560 mμ are replaced by two strong bands: α, 563, β, 533 mμ (α > β). In this reaction, of the two molecules of water co-ordinated with the divalent hæm iron in positions 5 and 6, only one is replaced by a molecule of carbon monoxide. Similarly, when a hæmochromogen is treated with carbon monoxide, one of the two molecules of the base is replaced by carbon monoxide, giving the corresponding carboxy-hæmochromogen[19] which, as was shown for the first time by Krebs[22], is sensitive to light.

The study of carbon monoxide derivatives is of great importance when considering certain reactions between hæm and proteins. In fact, as carboxy-hæm has a much greater affinity for nitrogenous substances than has free hæm, it was used as well as mono-cyan hæm for the final assessment of compounds as to their ability to combine with the iron of hæm. Moreover, since carboxy-hæm, like mono-cyan hæm, can combine with only one nitrogenous group, it can be used for the study of the reactivity of such a group when, owing to some spatial considerations, its co-ordination with the hæm iron is possible on only one side of the flat hæm molecule.

Hæmochromogen Formation by Amino-acids and Denatured Proteins

The reactions of free hæm with amino-acids and proteins are of special interest for the study of the hæm-binding groups in hæmoglobin as well as in other natural hæmoproteins. It can be demonstrated spectrophotometrically that neither free hæm nor free hæmatin combines with hæmoglobin or methæmoglobin respectively, which indicates that all the available hæm-binding groups of the native globin are already occupied. On mild denaturation, hæmoglobin forms globin hæmochromogen, which resembles the hæmochromogens given by hæm with different nitrogenous bases. When, however, free hæm is added to globin hæmochromogen, the absorption bands of the latter are greatly intensified, showing that denaturation of the globin increases its hæm-binding capacity at least six-fold. Although this property of globin, as well as of other proteins, was known for a long time[24–29], its full significance has not yet been properly appreciated. It was tacitly assumed that denaturation of proteins unmasks several potential hæm-binding groups belonging to different amino-acid residues, but early attempts to demonstrate the correctness of this assumption, by studying the reactions of hæm with free amino-acids, were very disappointing. According to Haurowitz[12], neither parahæmatin nor hæmochromogen could be obtained from hæmatin or hæm, respectively, treated with histidine, alanine, leucine, glycine, cysteine or glutathione, although it had been previously recorded[25] that glycine does give a hæmochromogen compound with hæm. Potentiometric titration[30] of 10^{-4} *M* hæmatin with sodium dithionite in the presence of histidine has shown that the oxido-reduction potential became constant only when the histidine added reached a concentration of 7×10^{-3} *M*. No attempt was made in this experiment to follow the reaction between hæm and histidine spectrophotometrically.

More recently spectrophotometric studies of the reactions between different hæms (protohæm, mesohæm, deuterohæm and urohæm) and amino-acids[16,27] revealed that, given the right conditions, typical hæmochromogen compounds were obtained with practically every amino-acid except proline, which shows that the α-amino group is a hæm-binding group common to all the amino-acids, provided that the hydrogens of the amino-group are not substituted; thus, while alanine gave a hæmochromogen, no hæmochromogen was obtained with N-monomethyl alanine or N-dimethyl alanine.

For the study of the reactions between hæm and proteins it was important to evaluate the hæm-binding properties of other potentially reacting groups of the amino-acids. Such evaluations were obtained from (1) the reactions of hæm with amino-acids in which the participation of the α-amino group was eliminated by being involved in a peptide linkage, or (2) the reactions of hæm with the potentially hæm-binding group either in the free state—for example, imidazole—or forming part of a compound free from other possible reacting groups—for example, the imidazole ring in pilocarpine.

The results of this work (unpublished), carried out with different hæms and their carbon monoxide derivatives, showed that only basic nitrogenous groups of amino-acids can combine with hæms or carboxy-hæms to give the corresponding hæmochromogens or their carboxy derivatives. This eliminates from the list of potential hæm-binding groups in denatured proteins the carboxylic side-chains of glutamic and aspartic acids, the benzene ring of tyrosine and phenylalanine and the sulphydryl group of cysteine. Of the nitrogenous basic groups such as the terminal α-amino group of different amino-acids, the ε-amino group of lysine, the imidazole group of histidine and the indole group of tryptophan, only the first three

were definitely established as potential hæm-binding groups. The hæm-binding property of the imidazole group of histidine was supported by the great ease with which free imidazoles, or the imidazole ring of pilocarpine, or of urocanic acid and certain other compounds form parahæmatins and hæmochromogens (unpublished work; see also refs. 18, 31, 32). Strong evidence for the hæm-binding property of the ε-amino group of lysine was obtained from the study of polylysine, which was found to have ten times higher affinity for hæm than free lysine. On the other hand, the side-chains of tryptophan and arginine were eliminated as potential hæm-binding groups by the failure to obtain hæmochromogens with free indole and free guanidine, as well as with denatured salmine and clupeine, which can be considered as polyarginine peptides. It was also found that whereas pyrrolidine gave a good hæmochromogen, free proline failed to co-ordinate with the hæm iron.

The potential hæm-binding groups, which become unmasked by the denaturation of proteins, are therefore limited to the terminal α-amino groups, the ε-amino group of lysine and the imidazole group of histidine. The total number of such groups[12] in whale and horse myoglobins (molecular weight, 17,000) are 32 and 28 respectively, and in horse hæmoglobin (molecular weight, 68,000) there are 76–80, that is, about 19–20 per unit of molecular weight, 17,000. Since the hæm-binding capacity or 'hæm-number' of sperm whale myoglobin is 10 and that of horse hæmoglobin is 6 per unit of protein of molecular weight 17,000 (ref. 27), and since each hæm iron co-ordinates with two nitrogenous groups to form a hæmochromogen, about 62 per cent of all available hæm-binding groups in denatured myoglobin and hæmoglobin are involved in hæmochromogen formation.

The study of hæmochromogen formation by denatured proteins including the globins, which will be fully dealt with elsewhere, has led to the following conclusions:

(1) As the iron atom in hæmochromogens is co-ordinated with two nitrogenous groups lying one on each side of the flat hæm molecule and occupying the fifth and sixth co-ordination positions, the hæm in denatured protein hæmochromogen must lie within the folds of the protein. This is made possible by the unfolding of peptide chains which characterizes denaturation in proteins, that is, the breaking of the bonds of the tertiary structure of the hæmoglobin molecule.

(2) The two nitrogenous groups, one on each side of the protein fold, which are linked to the hæm iron are not necessarily of the same nature; for example, one may be an imidazole group and the other an ε-amino group of lysine, or even an α-amino end-group.

(3) Since two nitrogenous groups, one on each side of the fold, are required for the formation of a hæmochromogen, there must be a certain regularity in their distribution in order to assume a high 'hæm number' which may involve more than 50 per cent of all the potential hæm-binding groups of the protein.

(4) The 'hæm number' of a denatured protein must therefore depend on: (*a*) the number of free nitrogenous hæm-binding groups; (*b*) a certain regularity in their distribution; and (*c*) freedom from steric hindrance by neighbouring side-chains. In this respect consideration should be given to all side-chains not involved in hæm-binding; for example, SH or S—S groups, the guanidine group of arginine, the indole group of tryptophan, the benzene ring of tyrosine or phenyl-alanine, and the carboxylic groups of glutamic and aspartic acids.

Reversible Formation of Hæmochromogen by Native Hæmoglobin

The fact that the iron atoms of hæmoglobin and methæmoglobin are accessible to, and easily combine with, a great variety of ligands some of which, like nitrosobenzene and imidazole, are of an appreciable size, clearly shows that the hæm or hæmatin is well exposed and not buried deeply within the folds of the protein[33–35].

That the hæm is bound to the globin not only by its iron atom but also by its porphyrin ring is indicated by the marked effect that free native globin has on the absorption spectrum of an alkaline solution of free porphyrin, and by the competition of porphyrin and hæm for native globin in experiments on the reconstitution of hæmoglobin from its components[36]. The two carboxyl groups of hæm are probably linked to the guanidine groups of the arginine residues[37].

Since the hæms in hæmoglobin are not buried between the folds of the globin, their iron atoms are linked to the globin by only one iron-protein bond (co-ordination position 5) lying on one side of the flat hæm molecule; on the other side (position 6), the iron is bound to a molecule of water, the presence of which was shown by spectroscopic studies[33] of the effect of low temperatures on alkaline methæmoglobin, and of the effect of drying on hæmoglobin and methæmoglobin.

The molecule of water in position 6 can be replaced in hæmoglobin by oxygen, carbon monoxide, nitric oxide, cyanide, alkyl cyanides or nitrosobenzene, and in methæmoglobin by fluoride, azide, cyanide, sulphide, nitric oxide or imidazole. As under certain well-defined conditions the denaturation of hæmoglobin was found to be a reversible process (unpublished work and ref. 38), it is possible that in the globin hæmochromogen thus obtained one of the two bonds uniting the hæm iron to globin is the original iron-protein bond present in hæmoglobin. The persistence of the original iron-protein bonds of hæmoglobin during the reversible formation of globin hæmochromogen and parahæmatin can be demonstrated by the following experiments.

(1) *Drying of hæmoglobin.* It has been shown that, when hæmoglobin or myoglobin is thoroughly dried, its absorption spectrum is replaced by that of a hæmochromogen[39–41]. On the addition of water, this reverts to hæmoglobin which, in the presence of oxygen, undergoes oxygenation to oxyhæmoglobin. This reversible formation of a hæmochromogen on drying is due to the fact that the molecule of water linked to the iron atom in the sixth co-ordination position is removed and replaced by a nitrogenous group of the protein belonging to the same molecule of hæmoglobin.

(2) *Drying of methæmoglobin.* In a similar way, the drying of acid methæmoglobin converts it reversibly to a parahæmatin[41]. That fluoride and azide methæmoglobin are not changed by drying to a parahæmatin is not surprising, since the fluoride or azide which replaced the water molecule of the methæmoglobin prevents the hæm iron from co-ordinating in position 6 with a nitrogenous group of the globin, brought closer to the iron by drying. The

fact that the absorption spectra of hæmoglobin and methæmoglobin are easily replaced on drying by those of the hæmochromogen and parahæmatin spectra, respectively, suggests that either an imidazole group of histidine or, more likely, an ε-amino group of lysine, must lie in close proximity to the water molecule attached to the iron atom.

(3) *The reaction of methæmoglobin with cyanide.* On treating acid methæmoglobin with cyanide, its colour changes from brown to red and its absorption band at 630 mμ is replaced by a wide band at 540 mμ. Cyan-methæmoglobin, which is one of the oldest known methæmoglobin derivatives[42], was at one time wrongly considered as an irreversible compound because of the very high affinity of cyanide for the tervalent iron of the methæmoglobin. Spectroscopically, it shows a close resemblance to the hybrid cyan-pyridine or cyan-imidazole parahæmatins referred to earlier.

(4) *The reaction of hæmoglobin with cyanide.* Cyan-hæmoglobin can easily be obtained by reducing cyan-methæmoglobin with sodium dithionite[43–45]. While the cyan-hæmoglobin obtained in this way is very unstable and rapidly dissociates into hæmoglobin and cyanide, cyan-myoglobin is sufficiently stable for spectrophotometric studies. It has a characteristic absorption spectrum very similar to that of cyan-pyridine or cyan-imidazole–hybrid hæmochromogens, with two strong absorption bands: α, 562 and β, 535 mμ (α > β). Cyan-myoglobin can be described as cyan-native globin–hybrid hæmochromogen, and its formation requires a high concentration of cyanide at *p*H 9. A compound which is practically indistinguishable from cyan-methæmoglobin was obtained[46] by treating reduced peroxidase with cyanide at *p*H 8·5–9·3. An interesting point was the discovery that cyan-ferroperoxidase and, to a lesser degree, cyan-myoglobin, undergo reversible photodissociation, liberating ferroperoxidase and myoglobin respectively and cyanide[46].

The fact that in these experiments hæmochromogens and parahæmatins were reversibly obtained from hæmoglobin and methæmoglobin respectively, on replacing the water molecule bound to the hæm iron in position 6 by a suitable nitrogenous compound, indicates that in position 5 the hæm iron must be linked to a nitrogenous compound which is the original hæm-binding group of the hæmoglobin. Since this group is a potential hæmochromogen-forming compound, it can only be the imidazole ring of histidine, the ε-amino group of lysine or, less likely, an amino end-group. This eliminates from the list of potential hæm-binding groups the side-chains of all other amino-acid residues, and also dismisses the possibility that the hæm-binding group could originally have been one of the non-hæmochromogen-forming side-chains which was replaced by a hæmochromogen-forming group only when the water molecule in position 6 was exchanged for a suitable nitrogenous substance. In fact, such a process would imply a rapid and reversible displacement of the hæm molecule in relation to the globin; this could scarcely take place, considering (*a*) that hæm is bound to the globin not only by its iron atom but also by the propionate groups and by van der Waals forces between its surface and that of the globin[16], (*b*) that reversible hæmochromogen formation may take place under conditions precluding the denaturation of the hæmoglobin, and (*c*) that cyan-native globin hæmochromogen can be dissociated by light into cyanide and hæmoglobin.

Evidence for the Imidazole Ring of Histidine as the Hæm-binding Group of Globin

Although the hæmochromogen criterion reduces the number of potential hæm-binding groups in hæmoglobin to only three types of basic groups, the imidazole ring of histidine, the ε-amino group of lysine and a terminal α-amino group, it makes no discrimination between them.

Of the views as to the nature of the hæm-binding group which were discussed earlier, the hæmochromogen criterion provides evidence only in favour of the imidazole theory which, as was shown above, was also supported by physico-chemical evidence[6–9], and by the facts that the globins of hæmoglobin and myoglobin are very rich in histidine. It must, however, be remembered that the physico-chemical evidence is largely based on the assumption that the properties of the imidazole ring of histidine which is incorporated in the peptide chain of the globin are quantitatively identical with those of the imidazole ring of free histidine. That this may not necessarily be so is indicated by the fact that the affinities of amino-acids for hæm are greatly enhanced when they form part of a peptide chain[18]. It has also been shown that the formation of a carbamino compound by glycine was increased thirty-fold by condensing two glycine molecules to a dipeptide[9]. Some caution must therefore be exercised in describing certain properties of proteins in terms of what is known of the individual amino-acids.

In spite of certain objections raised against the idea of the imidazole nature of the hæm-binding group in mammalian hæmoglobins and myoglobins[10–12,14,15,35], this theory, unlike any of the others so far proposed, is strongly supported both by the hæmochromogen criteria and by our present knowledge of the properties of hæmoglobin and its derivatives.

More direct evidence in favour of the view that the imidazole group of histidine binds the hæm iron to the globin was obtained from the X-ray analysis of crystals of myoglobin[47]. Indeed, the great advances which were recently made by Perutz and his co-workers[48] in their studies on the structure of hæmoglobin and by Kendrew and his co-workers[47,49] on the structure of myoglobin enabled them to unravel the tertiary structure of a protein molecule for the first time. Their results were based on three-dimensional Fourier syntheses at 5·5 Å. resolution in the case of horse oxy- and met-hæmoglobin[48] and at 2·0 Å. resolution in the case of sperm whale metmyoglobin[47,49].

The myoglobin molecule was found by Kendrew *et al.*[49] to consist of a single polypeptide chain folded in a somewhat complex manner, the straight portions of the chain having the structure of cylindrical tubes consisting of α-helices. The high degree of resolution has also shown that an amino-acid residue strongly suggestive of histidine, arising from the peptide chain adjacent to the hæm disk, is directed towards the hæm iron atom, whereas on the other side of the disk, what appears to be the oxygen of a water molecule lies very close to the iron. The terminal amino-group lies well away from the hæm.

The molecule of horse hæmoglobin was found by Perutz *et al.*[48] to be composed of four sub-units, each consisting of a single peptide chain folded in a very similar manner to that of sperm whale myoglobin. The hæm of each sub-unit is located in a pocket of the folded peptide chain, the four pockets being com-

Table 1. AMINO-ACID CONTENTS, ISOELECTRIC POINTS (I.P.) AND MOLECULAR WEIGHTS (MOL. WT.) OF SOME OXYGEN CARRIERS

Hæmoprotein oxygen carrier	Grams of amino-acid per 100 gm. protein*						I.P.	Mol. wt.
	Hist.	Lys.	Arg.	Trypt.	Tyr.	Cyst.		
Myoglobins								
Sperm whale (ref. 13)	11·12	15·90	2·24	2·60	2·12	0	6·78	17,000
Aplysia (marine mollusc) (ref. 50)	1·22	6·89	4·57	—	1·48	0	4·5	18,000
Hæmoglobins								
Horse (ref. 13)	8·71	8·51	3·65	1·70	3·03	1·01	6·9	68,000
Earthworm (ref. 51)	4·68	1·73	10·07	4·41	3·47	1·41	5·3	2,750,000
Arenicola (marine worm) (ref. 51)	4·03	1·85	10·04	1·64	2·52	4·08	4·5	2,750,000

* The amino-acids selected are limited to those which have been determined in certain invertebrates (ref. 51). In the cases of mammalian hæmoglobin and myoglobin (ref. 13) and *Aplysia* myoglobin (ref. 50), the complete balance sheet is available.
Abbreviations: Hist., histidine; Lys., lysine; Arg., arginine; Trypt., tryptophan; Tyr., tyrosine; Cyst., cysteine.

pletely independent of each other, the iron atoms of the closest pair being 25 Å. apart. The hæm iron on the side where it is linked with a water molecule, both in myoglobin and in hæmoglobin, is freely accessible to ligands of a large size[32] including *p*-iodophenylhydroxylamine[48].

The finding that the molecule of horse hæmoglobin consists of four sub-units each with a structural configuration closely resembling that of sperm whale myoglobin is very interesting and unexpected, considering that the amino-acid compositions of these two hæmoproteins show marked quantitative differences in the case of practically every amino-acid residue[13]. Some of these differences are shown in Table 1.

It was suggested by Perutz *et al.*[49] that "probably the chain, once it is synthesized and provided with a hæm group around which it can coil, takes up this configuration spontaneously, as the only one which satisfies the stereochemical requirements of its amino-acid sequence". This suggestion has direct bearing on the problem of the susceptibility of hæmoglobin to denaturation, which consists primarily in the breaking of bonds leading to the opening or uncoiling of the tertiary structure. Of special importance in this respect are the observations on the reversibility of denaturation in hæmoglobin[36]. It is also known that globin separated from hæm is very unstable, and that the susceptibility of hæmoglobin to denaturation is also affected by the nature of the ligands occupying co-ordination position 6 and by the nature of the bonds which link the hæm iron to surrounding atoms. Thus oxyhæmoglobin is much more resistant than methæmoglobin to denaturation by strong alkali or chloroform. Finally, hæmoglobin is more susceptible to irreversible denaturation than myoglobin. This may be due to the close proximity of the four sub-units in hæmoglobin which, in the partly uncoiled state, may interfere with each other in the proper recoiling of the peptide chain.

Whether or not the structural configuration of the myoglobin molecule is of general significance will be determined only by the study of invertebrate hæmoglobins and myoglobins, the amino-acid balance sheets of which show very great differences from those of the mammalian hæmoproteins[12,50,51] (Table 1). The myoglobin of the marine mollusc *Aplysia* is of special interest in this respect. *Aplysia* myoglobin (molecular weight 18,000), which has been obtained in a pure crystalline state, contains only one histidine residue per molecule, and it differs from mammalian myoglobins and hæmoglobins in the content of all the other amino-acid residues[50]. Considering that, apart from *Aplysia* myoglobin, investigations have been carried out on the hæmoglobins of only very few invertebrates and on material which was incompletely purified and scarcely sufficient in quantity for accurate analysis by the methods then used, it is possible that further work may reveal the existence of a hæmoglobin devoid of histidine. That histidine is not a universally distributed amino-acid is shown by its absence from the proteins of certain strains of viruses[52]. The hæmochromogen criterion has, however, shown that in a hæmoprotein deficient or completely free of histidine, the hæm-binding role of the imidazole ring could be taken over by the ε-amino group of lysine or even by a terminal α-amino group.

Indeed, it is only the study of hæmoglobins and myoglobins of different origins, which show the greatest diversity in their amino-acid compositions and in their physical and chemical properties, that will reveal the nature of the structural peculiarity common to all of them (but not to other hæmoproteins which only undergo oxido-reduction) that confers on the divalent iron of the hæm the property of combining reversibly with molecular oxygen without undergoing oxidation.

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NEWS and VIEWS

Physiology at University College, London: Prof. A. F. Huxley

Mr. A. F. Huxley, who was recently appointed to succeed Sir Lindor Brown (*Nature*, **185**, 733; 1960) as Jodrell professor of physiology at University College, London, is well known internationally for his very distinguished research record. Among his contributions may be mentioned the important part he played in elucidating the mechanism of the nerve impulse and in proving the 'saltatory' nature of propagation in myelinated axons. More recently, he turned his attention to the microscopic structure of muscle fibres and was able to clarify greatly the sequence of the structural changes which occur in striated muscle during activity. Throughout his experimental work, he has tackled difficulties with a remarkable technical ingenuity and mathematical skill, developing important new instruments as well as theories as he went along. A. F. Huxley started at Cambridge and Plymouth, in collaboration with Prof. A. L. Hodgkin, a partnership which proved extremely fruitful and continued for many years. During the Second World War, Huxley was engaged in operational research, first with Anti-Aircraft Command and later with the Admiralty. In 1941, he became a Fellow of Trinity College; his most recent Cambridge appointment was to a readership at the Physiological Laboratory. He was elected to the fellowship of the Royal Society in 1955. Although Huxley's research work is highly specialized, his scientific interests cover an unusually wide range, and many physiologists feel indebted to him for the help they received during the period of his editorship of the *Journal of Physiology*. Those who visit the meetings of the Physiological Society regularly are well aware of Prof. Huxley's ability to penetrate scientific problems of a diverse nature. No doubt, in his new appointment, he will find plenty of opportunities of applying this gift.

The Overseas Civil Service

In an adjournment debate on the Overseas Civil Service on July 11, Mr. B. R. Braine, Mr. J. Callaghan, and Mr. J. D. Tilney pressed the claims of the Overseas Civil Service. Mr. Julian Amery, Under-Secretary of State for the Colonies, who replied for the Government, said that the strength of their plea was fully justified by the importance of the subject and the urgency of the problem. He agreed that strong, efficient and honest administration had been Britain's greatest contribution to the development of the colonies and that one of our main tasks is to leave a strong local administrative service. This is partly a matter of establishing proper principles governing the local administrative services, of taking them out of politics, and of making appointments the responsibility of public service commissions. It is also a matter of retaining the services of ex-patriates for many years to come, and particularly of discovering ways and means of encouraging them to stay and go on serving now that the Secretary of State, as power is transferred, can no longer offer assurance of a career structure and promotion prospects or guarantee jobs in Britain. There must instead be special inducements to serve, and special compensation for loss of office, if and when it comes. At present there are 15,000 pensionable members of the Overseas Service, including 7,000 in East Africa, 1,500 in Nyasaland and Rhodesia, and 2,000 in West Africa, and in addition more than 10,000 are on contract. It is our duty, he said, as well as our interest, to see that, as these territories move to independence, they have an administrative structure which will enable them to maintain stability and to achieve some economic and social expansion. Mr. Amery said he could not make a statement that night, but he assured the House that the Secretary of State for the Colonies regards the problems raised as of the highest importance and calling for urgent solutions.

British and Foreign School Society and Borough Road College

The Nuffield Foundation has given £50,000 to the Borough Road College, Isleworth, for a new science block which will house mathematics, biology, chemistry and physics in one building. It will be known as the Nuffield Science Building. This independent College has a long tradition of producing teachers of mathematics and science, and the Foundation has made the grant to enable it to increase the number of specialist teachers of these subjects. Borough Road College belongs to the British and Foreign School Society, which is contributing £75,000 towards the general expansion of the College. With the aid of a capital grant from the Ministry of Education, it will be able to raise the number of students from 320 to 500. The College is one of the oldest teacher-training colleges in the world, and recently celebrated its 150th anniversary.

Science and the Public

In his foundation oration, "The Diffusion of Knowledge", at Goldsmiths' College, University of

London, on March 17, Dr. Michael Grant, president and vice-chancellor of the Queen's University of Belfast, discussed particularly the extraordinarily increasing activity and problem of making specialized knowledge available to the non-specialized adult public, and especially—since his subject was the diffusion of knowledge—of the printed word rather than the use of television or sound-radio (Pp. 15. London: Goldsmiths' College, 1960). The amount of actual knowledge imprinted on the adult general public by these media remains infinitesimal in comparison with what is gathered from printed books; and Dr. Grant directed attention to the fact that commercial advertisers still spend only 13 per cent of their funds on television and 47 per cent on the Press. The sale of informative paper-backed books, of which first editions of as many as 50,000 might be printed and sold, was further evidence of the existence of a demand for popular knowledge.

Discussing the problems involved in popularization, Dr. Grant referred to the difficulty of simplification but emphasized that good popularization could create the atmosphere for good scholarship, in which before long important new research could arise: diffusion and stimulus could go together. There were also problems of vocabulary and presentation as well as of scholarship, nor was it possible to ignore entirely pronouncements on moral or even social values. Even a smattering of reasonably accurate knowledge, however, would make the non-specialized adult population much less likely to become credulous victims of all the nonsense of which people try to convince them, and the spreading of knowledge by popularization was highly relevant to the problem summed up by Sir Charles Snow as that of the two cultures. That problem could not be left entirely to the universities, though he thought they should study deliberately how to produce the habit of mind which will make its students want, both before and after graduation, to take an open-minded interest in at least something worth while outside their own careers. In conclusion, he appealed to his hearers generally to participate themselves in this extremely difficult, heavily criticized but fascinating task of popularizing knowledge, which was worth doing and had to be done, and was more likely to be done successfully by teachers than by most other people.

New C.S.I.R.O. Sugar Research Laboratory

It is announced that a new Sugar Research Laboratory will be set up by the Commonwealth Scientific and Industrial Research Organization, to form part of the Organization's Chemical Research Laboratories in Melbourne. The decision to establish the Laboratory has come after considerable discussion with representatives of the sugar industry, which is anxious to find new uses for the present surplus in Australian production. The surplus corresponds to about a million tons of sugar cane per year. Australia's biggest sugar producer, the Colonial Sugar Refining Co., Ltd., has agreed to contribute £2,500 per annum towards the running costs of the Laboratory. Dr. H. H. Hatt, previously officer-in-charge of the Organization's Organic Chemistry Section, has been appointed leader of research in the new unit.

The Output of Scientists in Scotland: 1600–1950

In a paper, "The Output of Scientists in Scotland, 1600–1950", published in *The Eugenics Review*, July 1960, R. H. S. Robertson advances evidence that from the beginning of the seventeenth until the middle of the eighteenth century the proportion of scientists born in Scotland who emigrated steadily increased: from then until about 1825 it tended to decrease, and in that year 71 per cent of the scientists selected for his sample died in Scotland, compared with 48 per cent in 1750. From 1825, however, the proportion of scientifically gifted Scots who emigrated has increased at an alarming rate, in spite of a slight evening off in the curve and even a recovery in recent years. Since a high proportion of the net loss of emigrants from Scotland of about 24,000 a year is from the more intelligent classes, Robertson urges that action is imperative if disaster is to be prevented. Scotland, he claims, produces 20 per cent of the British output of scientists and technologists, but only some 3 per cent of British scientific posts are available within her boundaries. Attributing the decline in the number of Scottish posts chiefly to the unequal distribution in Britain of research institutions, he urges that research institutes and posts in Scotland should be increased to about 10 per cent of the British total.

Museum of Applied Science of Victoria, Melbourne

The annual report for 1958–59 of the Museum of Applied Science of Victoria, Melbourne, records the welcome news that tenders have been accepted for building extensions and that it is hoped to complete the work by the end of 1960. As available space will be increased by about 50 per cent, this will be the greatest expansion of the Museum since its foundation in 1870 (Pp. 30. Melbourne: Museum of Applied Science of Victoria, 1960). Major displays include exhibitions celebrating one hundred years of State-operated railways in Victoria, present-day telephones, and a sectioned model of a reactor. The increasing interest in space exploration and upper atmosphere research led to the installation of a working exhibit showing satellites in orbit around the Earth. A working exhibit was also made to demonstrate the behaviour of a low-voltage halogen-quenched Geiger tube and its use for radiation measurement. The circuit includes a counting-rate meter for visual monitoring and a loud-speaker for aural monitoring.

Indexes for Scientific and Technical Reports

An investigation by the Research Department of the Association of Special Libraries and Information Bureaux into the indexing of published reports of scientific and technical conferences and symposia, prompted by an inquiry which cited some examples of unindexed publications of this type, established that half the published proceedings and reports of such conferences are provided with neither subject nor author indexes. Based on a survey of some 205 such publications noted in the British National Bibliography during January 1956–September 1959, inclusive, the survey showed that 59 per cent were without subject indexes and 66 per cent without author indexes, 50 per cent being without both. Publications of 301–400 pages were best provided with subject indexes (66 per cent), whereas 90 per cent of those of up to 100 pages were without, 71 per cent of those with 101–200 pages, 57 per cent of those with 201–300 pages, 54 per cent of those with 401–500 pages and 50 per cent of those with more than 500 pages. The position appears to be worsening, the percentage of publications without subject indexes in 1956 being 47; in 1957, 60; 1958, 61; and in

1959, 68, although the latter year is incomplete. A breakdown into broad subject groups showed that for biological and allied sciences the percentage without subject indexes was 53, and for physics, chemistry, engineering and technology, 60.

The report also notes the considerable time-lag between the holding of a meeting and publication of the report of its proceedings, and points out that publications which took more than two years to prepare were even less likely to contain subject indexes than those issued in the same year as the meeting. About a quarter of the reports were published by the organizers of the conferences themselves, and of these 79 per cent were without; about three-fifths of the remainder were issued by four publishing houses, and for these the percentages without subject indexes were 59, 96, 48 and 33. For the 22 reports issued by commercial publishers, the percentage without subject indexes was 35 per cent, and it is worth noting that all ten issued by one and seven out of eight issued by another had subject indexes. The risk that information sought in such reports may not be found is further illustrated by a more detailed example of a random sample of five publications, but this limited survey could not attempt to examine how far the contents of such reports are published elsewhere or caught up by abstracting organizations.

Leo III

An impressive addition to the field of the automatic office is *Leo* III, a new all-transistor digital computer under development by Leo Computers, Ltd. The machine, which incorporates many of the latest advances in data-processing techniques, makes use of a micro-programming construction and can perform arithmetical operations directly on decimal, sterling or any other desired quantities. The time taken for a typical decimal addition is 44 μsec. Perhaps the most interesting feature is that it is one of the first British computers to incorporate an automatic time-sharing facility. This enables two or more different programmes to be run on the computer at the same time and assists in obviating periods of waiting by (say) the arithmetic unit during the relatively slow operation of the peripheral equipment. Another feature is the large high-speed magnetic core store, which has a capacity of up to 32,768 words of 44 bits each. Further storage is provided by magnetic tape, each tape being capable of holding several million alpha-numeric characters, with a peak transfer speed of 90,000 characters per sec. A wide range of input and output devices is also available, and these include a line-printer with a maximum speed of 850 lines per min.

Radio Frequencies and Space Communications

The increasing demand for frequencies for various applications of radio technique has led to a state of congestion in the radio-frequency spectrum. At its autumn meetings in Geneva, the International Telecommunications Union gave earnest consideration not only to the more orthodox uses of radio-waves, but also to the requirements of radio astronomy and space research; and these must include in the near future the frequencies to be used for communications with and between vehicles in space. For the information of the United States Senate Committee on Aeronautical and Space Sciences, a report, by Edward Wenk, jun., has recently been issued under the title "Radio Frequency Control in Space Telecommunications" (United States. 86th Congress 2nd Session. Legislative Reference Service–Library of Congress. Pp. vii+235. Washington, D.C.: Government Printing Office, 1960). This report explains the dependence of the exploration of space on radio communication, reviews briefly the technical considerations which determine the selection of frequencies for various purposes, and summarizes the history of international agreements on frequency allocation. An outline is given of the organization of the International Telecommunications Union, with its technical advisory body, the International Radio Consultative Committee, and its relationship with the International Council of Scientific Unions, to which are affiliated the International Scientific Radio Union and the Committee on Space Research.

While most of the report is concerned with problems domestic to the United States in the allocation and control of radio frequencies for all purposes, some extensive appendixes include the report of the *ad hoc* Committee on the Peaceful Uses of Outer Space adopted by the United Nations General Assembly in 1959. There is also a world-wide summary of frequencies recorded by the International Frequency Registration Board of the International Telecommunications Union, and a copy of the Radio Regulations and Frequency Allocations adopted by the Administrative Radio Conference of the Union at Geneva in 1959. These appendixes make the U.S. report a very useful work of reference to those interested in international radio matters.

Tectonics of the Gobi Mountains

The aerial and land survey of the fault and fissure zone in the Gobi mountain range, as resulting from the earthquakes of December 7, 1957, and April 7, 1958, is discussed by V. P. Solonenko and N. A. Florensov (*Priroda*, **2**, 61; 1960). The disturbed zone stretches almost parallel to latitude 45° N., and extends for almost 300 km. from longitude 99° E. to 102° 20′ E. The zone consists of a number of faults, one with a visible throw of 328 m., enclosing several horsts and rift-valleys, one rift-valley having dimensions 27 km. long and 800 m. wide.

Newly Discovered Rock Shelter Art in Malaya

Why mankind should want to draw and paint in caves and rock-shelters cannot as yet be determined. Probably the reasons are multiple. In the case of palæolithic man, it is likely that sympathetic magic in connexion with food supply was the motive, but such an urge cannot be adduced in the case of the South and Central African rock-shelter art, nor in the case of some newly discovered decorated rock-shelter walls near Ipoh, Perak, Federation of Malaya. These latest discoveries were made by Lieut. R. L. Rawlings, of the Gurka Rifles, and are described in *Man* for January 1960 by J. M. Matthews, curator of museums, Federation of Malaya. Animals, men and designs are depicted in various shades of hæmatite (red), and at least three styles can be distinguished. Most of the caves in Malaya have been dug for cave earth and guano, so there are few undisturbed deposits. No correlation of this new-found art with any known culture is possible. Comparison of drawings made in one area with others elsewhere on stylistic grounds is more than dangerous; most of these drawings are essentially simple, and any conventionalizations are what one might expect to occur anywhere independently. Some day the age of this Malayan art will become known, and also why it

was made. In the meanwhile, one must be grateful for the excellent account here given.

The First Forty Years of the Royal Society of London

An exhibition has been arranged at the Science Museum to illustrate the first forty years of the Royal Society of London. The exhibition is mainly biographical, and illustrates the scientific life and work of some twenty men, who were distinguished Fellows during the period 1660–1700. The exhibits consist of a collection of portraits (enlarged photographs of engravings), copies of the Fellows' scientific publications, photographs of early documents of the Society, and a small collection of instruments and apparatus.

British Men of Science

To coincide with the tercentenary of the Royal Society, the preparation of a new series, *British Men of Science*, is announced by Nelson under the general editorship of Sir Gavin de Beer. The series will be designed to provide authoritative but short lives, written by leading scientists and intended for the general reader and university student. So far, twelve volumes are in preparation, but others are being actively planned. They are: "Joseph Black", by Prof. Douglas McKie; "Charles Darwin", by Sir Gavin de Beer; "Humphry Davy", by Sir Harold Hartley; "Edmond Halley", by Sir Harold Spencer Jones; "William Gilbert", by N. H. de V. Heathcote; "William Herschel", by Angus Armitage; "Charles Lyell", by Sir Edward Bailey; "William Henry Perkin", by Prof. John Read; "Joseph Priestley", by F. W. Gibbs; "Ernest Rutherford", by Sir Ernest Marsden; "Michael Scot", by Prof. Lynn Thorndike; "Joseph John Thomson", by Sir George Thomson. The series, which will be fully illustrated in line and half-tone, will contain a list of books for future reading.

"Non-Enzymic Reactions of Indoles with Coenzyme I"

Prof. S. G. A. Alivisatos, G. A. Mourkides and A. Jibril write: "In a previous communication (*Nature*, **186**, 718; 1960) we described certain interactions occurring between indoles and coenzyme I. While recent evidence obtained in our laboratory with a number of other indoles and with the reduced form of coenzyme I confirmed our original observations, pointing towards an interaction of the indole with the nicotinamide moiety of the oxidized form of coenzyme I (probably, a charge transfer complex), the previously reported decrease in absorbancy observed in the ultra-violet region below 310 mμ was later shown to be an artefact produced by stray light when one of the reactants was present at high concentration in the blank".

Announcements

Prof. P. B. Medawar, C.B.E., F.R.S., Jodrell professor of zoology and comparative anatomy at University College in the University of London, has been appointed director of the National Institute for Medical Research, Mill Hill, London, as from August 1962. Prof. Medawar succeeds Sir Charles Harington.

The following have been elected honorary members of the American Institute of Nutrition: Lord Boyd Orr, Drs. W. A. Aykroyd, F. B. Berry, F. R. Boudreau, R. C. Burgess, F. W. Clements, Dr. D. P. Cuthbertson, V. N. Patwardhan, Prof. E. F. Terroine and Prof. A. I. Virtanen. This is the first occasion on which the Society has elected honorary members.

Dr. J. H. Gerber has been appointed director of the Center for Aging Research of the National Institutes of Health, U.S. Department of Health, Education and Welfare, in succession to Dr. G. Halsey Hunt, who is now chief of the Division of General Medical Sciences, of which the Center is a component.

Dr. J. J. Gilman, of the Research Laboratory, General Electric Co., Schenectady, is to give a lecture on "The Physical Nature of Plastic Flow", under the auspices of the Metal Physics Committee of the Institute of Metals. The meeting will be held at the Institute's headquarters, 17 Belgrave Square, London, S.W.1, on Tuesday, August 16, and from which further information can be obtained.

The Belgian Society of Pharmaceutical Sciences is organizing an International Symposium on Chromatography in Brussels during September 16–17. Further information can be obtained from the Belgisch Genootschap voor Pharmaceutisches Wetenschappen, 11 Archimedesstraat, Brussels 4.

The Universities Federation for Animal Welfare is holding a Summer Congress at Culham College, Abingdon, Berks, during September 16–22. Further information can be obtained from UFAW (Summer Congress), 7*a* Lamb's Conduit Passage, London, W.C.1.

A non-residential summer school on "Numerical Analysis" will be held in Oxford during September 26–October 7. The object of the course is to teach the elements of numerical analysis in the fields of linear algebra and differential equations to users of automatic digital computers in industry, government and university departments. Further information can be obtained from the Secretary to the Delegacy for Extra-Mural Studies, Rewley House, Wellington Square, Oxford.

The fifth International Instruments and Measurements Conference and Exhibition are to be held in Stockholm during September 10–17. The Conference is organized by the Royal Swedish Academy of Engineering Sciences and the Swedish Association of Technical Physicists. Further information concerning the British participation can be obtained from the Chief Press Officer, U.K. Atomic Energy Authority, 11 Charles II Street, London, S.W.1.

The first British Congress on "The History of Medicine and Pharmacy" is to be organized by the Faculty of the History of Medicine and Pharmacy at the Worshipful Society of Apothecaries of London, in co-operation with the Royal Colleges and the Royal Society of Medicine, and held in London during September 29–30. Further information can be obtained from Dr. F. N. L. Poynter, Wellcome Historical Medical Library, The Wellcome Building, Euston Road, London, N.W.1.

As from July 31, the Imperial College of Tropical Agriculture is to be merged into the University College of the West Indies, and the College will be known as the University College of the West Indies, Faculty of Agriculture (Imperial College of Tropical Agriculture), Trinidad, West Indies. The London office of the College is to be closed as from August 31.

DOCTOR RUGGLES GATES AND THE ABORIGINAL AUSTRALIAN

By Prof. A. A. ABBIE
Department of Anatomy, University of Adelaide

DR. R. RUGGLES GATES has recently published two papers on the Australian aborigines. One deals with some genetical aspects of 'race' crossing[1], the other with the homogeneity or otherwise of the aborigines[2]. The first reaction to these papers is of profound admiration that so much could result from so brief a visit and that such far-reaching conclusions could be extracted from such scanty material.

I am not a geneticist but over many years have had close personal contact with large numbers of aborigines in most parts of the continent and it is against that background that Dr. Gates's contributions are considered here.

In the first place it is necessary to point out that Dr. Gates is correct in many of the statements he makes—this is when he is repeating the findings of earlier workers. His paper on the genetics of interbreeding[1] is beyond my competence except to express surprise at the confidence with which Dr. Gates supplies pedigrees when, in truth, aboriginal marriage customs and beliefs render the compilation of a genealogical tree almost entirely a matter of faith (in this connexion, see Abbie and Adey[3]). His second paper is concerned mainly with full aborigines and raises a number of points (some are common to both papers) that deserve further consideration. The most important relate to pigmentation, hair-form, stature and head-form and they will be reviewed in that order.

Aborigines are born pinkish yellow (not white) and darken in a few weeks or so according to the degree of solar exposure. We have never seen a sacral ('mongolian') spot. Darkening of the skin usually stops at a warm medium vandyke, but it may darken further on intense exposure in the desert or in the extreme north. Protection from the Sun, as in fat creases, but more particularly when clothing is worn habitually, causes the skin colour to fade—sometimes almost to the original pinkish yellow—and in the latter case undue exposure may lead to quite severe sunburn[3,4]. Dr. Gates is evidently aware of this work yet he attributes a yellowish shade in some northern Queensland natives to 'Tasmanian' affinities, a fair example of ignoring facts to push a hypothesis. In central Australia particularly, but also in South Australia, many of the children are fair-headed, the colour ranging from completely flaxen to almost rufous brown and darkening with age[3–5]. Dr. Gates appears to support Birdsell's[6] view that this condition appeared first in the south-west of central Australia and is spreading more or less concentrically outwards and especially westwards. In fact, the condition was first described in Western Australia (at New Norcia) in the middle of the last century, and Griffith Taylor's original observations on fairheadedness were also made in Western Australia. We have noted fairheadedness on the south coast at Fowler's Bay; in central Australia it is widespread but with a patchy distribution that does not support the conception of continuous centrifugal spread. But we have also found fairheadedness (though less frequently) in the extreme north of Arnhem Land where the possibility of spread from central Australia is practically zero. In any event, since intertribal breeding was, and still is, virtually unknown except recently on Government Settlements, what mechanism can be adduced to account for the spreading?

Dr. Gates puts great reliance upon the occurrence of curly or frizzy hair (associated with short stature) to establish the 'negrito' character of some northern Queensland aborigines. Photographs of these people published by Tindale and Birdsell[7] reveal hair-forms from practically straight to deeply curled, and that is what is found normally in all aboriginal groups anywhere in the continent[4,5]. In the extreme north curly-to-woolly hair is commoner than elsewhere but there is some evidence of Indonesian and Melanesian admixture (including blood group B). Also, Dr. Gates does not take into account (although he is aware of the fact) that in the last century some thousands of male 'kanakas' of Melanesian origin were imported into northern Queensland to harvest sugar cane. They were afterwards repatriated but scarcely, one imagines, without leaving their mark on the local aboriginal population. The most tightly curled hair seen by me belonged to a Pitjantjara male in central Australia, about as far away from the coast in any direction as it is possible to get.

Stature Dr. Gates considers diagnostic of the 'negritos' of northern Queensland. For 9 Monamona males he gives a range of 141·5 cm.–161·5 cm., with a mean of 155·8 cm. However, the shortest is an acknowledged dwarf: without him the range is 150·0 cm.–161·5 cm., with a mean of 157·6 cm. Our (unpublished) figures, based upon about 30 adult males in each case, for four areas across the continent are: south (Fowler's Bay): 146·0 cm.–174·0 cm. (mean 163·1 cm.); centre (Pintubi): 152·0 cm.–180·7 cm. (mean 167·1 cm.); (Wailbrai): 159·5 cm.–180·1 cm. (mean 170·3 cm.); northern Arnhem Land (Burera): 150·4–187·3 cm. (mean 169·6 cm.). Clearly there is an enormous overlap and it seems at least possible that had Dr. Gates examined a larger sample he would have been less positive about the pygmoid character of the northern Queensland aborigines. The shortest adult male aborigine so far recorded[8] measured only 135 cm.; he was found in the Peterman Range near central Australia, but short people occur anywhere. Dr. Gates believes that half-castes show heterosis, being larger, better formed and more fully developed; this we cannot confirm.

The shape of the skull is another factor Dr. Gates adduces to support his views on the 'negrito' character of north Queensland aborigines. The range of cephalic indices in his series of 9 was 70·56–81·76, with a mean of 74·48 (the highest index came from the dwarf previously mentioned). Corresponding figures for our groups were: Fowler's Bay: 68·2–79·2 (mean 73·3); Pintubi: 66·2–81·4 (mean 71·5);

Wailbrai: 67·4–84·3 (mean 73·5); Burera: 64·2–80·0 (mean 70·9). Obviously, while the ranges are wider, some of these means are very close to the northern Queensland mean, and many of these heads are much more brachycephalic. Indeed, if the dwarf were eliminated the Monamona mean would not differ materially from any other aboriginal mean. One further point: while Dr. Gates tacitly endorses my view[9] that head-length and head-breadth vary independently, he evidently did not read far enough to gain some insight on brachycephaly. It is true that, *ceteris paribus*, head-length is determined genetically (subject to some environmental modification as Boas has shown) in fully developed adults, but it is not necessary to invoke any involved genetic mechanism to explain short-headedness in pygmoid people. These are in an infantile state of development and their heads betray the characteristically infantile brachycephaly.

There is no space here to mention a number of factual errors in Dr. Gates's papers, and certainly no necessity to follow his excursions into archæology and geology. Perhaps it is best to conclude with one's own views, based upon the examination of several hundreds of aborigines in various, widely separated, parts of the continent.

Aborigines betray a fairly wide range of physical variation, but such variation is intratribal, not intertribal. That is, the tribes, practically anywhere, show a parallelism in the range of variation, and the means for the tribes come out remarkably close to each other. This implies a high level of physical homogeneity throughout the continent—all the more singular because the maximum aboriginal population is said never to have exceeded some 300,000 and such a small number, dispersed in small inbreeding groups throughout an area of nearly 3,000,000 square miles, surely had little chance of achieving such uniformity by interbreeding. On the contrary, genetic drift in what were practically isolates would be expected to produce a wide physical diversity from one end of the continent to the other. The fact that what diversity occurs is but slight points to a few tentative conclusions. It suggests first that the initial aboriginal landing comprised only a relatively small party bringing only a limited genetic pool. The second conclusion is that there had been a minimum of subsequent admixture with other peoples before the advent of Europeans. Thirdly, the aborigines could have occupied Australia for only a relatively short period—certainly not long enough to produce any obvious physical differences from place to place. This last is in harmony with their uniformly mesolithic culture[5] and with carbon-14 datings which, so far at least, cannot certainly be associated with human occupation beyond about 6,000 B.C.

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[3] Abbie, A. A., and Adey, W. R., *Amer. J. Phys. Anthrop.*, **11**, 339 (1953).
[4] Abbie, A. A., *The Leech*, **28**, 120 (1958).
[5] Abbie, A. A., *Oceania*, **22**, 91 (1951).
[6] Birdsell, J. B., *Cold Spring Harbor Symp. Quant. Biol.*, **15**, 259 (1950).
[7] Tindale, N. B., and Birdsell, J. B., *Rec. S. Aust. Mus.*, **7**, 1 (1941).
[8] Basedow, H., "The Australian Aboriginal" (Preece, Adelaide, 1925).
[9] Abbie, A. A., *J. Anat.* (Lond.), **81**, 233 (1947).
[10] Abbie, A. A., *Oceania*, **27**, 220 (1957).
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HERBICIDES AND THE SOIL

MODERN techniques of herbicidal applications fall into two groups. Direct application may be made to the soil followed by absorption through the roots or applications may be made to the foliage where entry is largely dependent on penetration through the leaves. This distinction is not, however, clear cut, since with foliar applications some of the droplets will fall on the ground while some of the compound may be washed off the leaves by rain. Thus for both types of technique it is of major importance to ascertain how long individual compounds persist in the soil, what are the physical or biological factors which cause their removal or decomposition and what are the effects on the fauna and flora of the soil. It was against this background that the British Weed Control Council organized a symposium on "Herbicides and the Soil", which was held in the Clarendon Laboratory, University of Oxford, on April 7.

The opening speaker was Prof. L. J. Audus (Bedford College, London) on "Microbiological Breakdown of Herbicides in Soils". Before direct investigations on the rate of decomposition of herbicides was undertaken it had been observed in the field that the persistence of some, as judged by the effects on plant growth, was minimal when the soil was moist and warm, conditions generally accepted as being favourable to micro-organisms. Since these conditions could also favour chemical decomposition it was difficult to separate the operative factors except in the laboratory. The fact that breakdown in the soil was largely arrested by autoclaving and that the addition of compounds known to be inhibitory to micro-organisms also slowed down the rate of decomposition pointed to the conclusion that micro-organisms were involved.

If micro-organisms are responsible for the breakdown of a herbicide, then the addition of such a compound to the soil under constant and favourable conditions will not result in immediate breakdown, as in a chemical process, but there will be a lag phase while there is a build-up of the population of the effective organisms.

At Bedford College the perfusion technique has been employed to study the breakdown of substituted phenoxyacetic acids and in every case the existence of a lag phase has been demonstrated. It has also been established that once decomposition has taken place in a soil, then the speed of breakdown of a further addition of the chemical is increased.

In 1950, Audus isolated an organism of the *Bacterium globiforme* group which was able to multiply on an agar medium containing 2,4-dichlorophenoxyacetic acid as the sole source of carbon. This organism, when added to garden soil, immediately accelerated the rate of breakdown of 2,4-dichlorophenoxyacetic acid. Since this original isolation, it has been found that a wide range of bacteria, actinomycetes and fungi are capable of using herbicides as their only source of carbon.

For the substituted phenoxyacetic acids there is no clear correlation between the duration of the lag phase and chemical structure, but it is apparent that the position on the ring of a single chlorine substituent is important; for example, a change of the substituent from the 4 to the 2 position makes the compound much more resistant to attack. In di- and tri-substituted compounds the presence of a 4-chloro substitution overrides the influence of other substitutions. N. Brownbridge, however, sought to discover whether ease of breakdown was linked with the level of phytotoxicity of the chemical to higher plants as judged by the concentration necessary to reduce the rate of growth of cress roots by 50 per cent. Excluding the value for 2-chloro-4 methylphenoxyacetic acid, there was a highly significant inverse correlation. The position of 2-chloro-4 methylphenoxyacetic acid was anomalous; the lag phase was far too long, judged on the degree of phytotoxicity.

If a soil was first treated with 2-chloro-4 methyl phenoxyacetic acid and another substituted phenoxyacetic acid was then added, the breakdown of the second compound was rapid; for example, even after prolonged perfusion 2,4,5-trichlorophenoxyacetic acid is very slowly decomposed but 2-chloro-4 methylphenoxyacetic acid treated soil can break down 2,4,5-trichlorophenoxyacetic acid more readily. Soils first treated with other phenoxyacetic acids did not gain the power to attack other compounds to the same extent.

Prof. W. W. Fletcher (West of Scotland Agricultural College, Glasgow) spoke on the "Effects of Herbicides on Soil Micro-organisms" and emphasized that although a few chemicals did reduce the population of the soil micro-organisms, the net effect was ultimately one of stimulation as one or more species became adapted to use the herbicide as a substrate. Organisms responsible for nitrification and nodulation appear to be more sensitive than other bacteria to a wide range of herbicides including sodium chlorate, 2-chloro-N,N-diethylacetamide and 2-chloro-N,N-diallylacetamide when applied at normal rates in the field. There is a general agreement that 2-chloro-4 methylphenoxyacetic acid, 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid at the normal rates employed in agriculture have no adverse effects on the total number of micro-organisms, and it would appear that 2,2-dichloropropionic acid augments rather than depresses the general soil population.

The species of one genus may vary greatly in their response to chemicals. For example, 3,5-dinitro-*o*-cresol inhibited *Rhizobium lupini* at 198 p.p.m., whereas *R. meliloti* and *R. leguminosarum* required 1,980 p.p.m. for inhibition.

Dr. G. S. Hartley (Chesterford Park Research Station) spoke on the "Physico-chemical Aspects of the Availability of Herbicides in Soils". Beginning with a simple theoretical model, Dr. Hartley defined his problem within the system—soil particulate structure–air–water–herbicide.

In the simplest case, if the water content is at 'field capacity' and a non-volatile water-soluble herbicide is applied uniformly to the soil surface, then as long as the system remains undisturbed the herbicide can move only by diffusion in the water phase. The rate of this movement can be calculated and for molecules of the size of herbicides 1 per cent of the applied chemical will move rather more than 2 ft. downward in three years. The presence of solid matter in such a system as well as air locks reduces the 'expected' rate of diffusion. When corrections for these obstacles are applied to the theoretical figure it is apparent that 1 per cent of an applied soluble herbicide might take twelve years to reach a depth of 2 ft. It must therefore be concluded that the downward movement of herbicides in the field is not by diffusion alone.

Again in an ideal uniform system, and assuming field capacity to be 25 per cent v/v, 10 cm. of rainfall would sweep a readily soluble herbicide to a depth of 40 cm., where it would be localized in a narrow band. In the field where a soil is heterogeneous the herbicide will move unevenly downwards. This unevenness is especially marked if rainfall is heavy, when the herbicide is washed down the larger channels or cracks in the soil. Dr. Hartley suggested that the difference between observed and calculated rates of movement could be largely explained in terms of the heterogeneity of the soil system.

Attempts to measure the rates of movement of herbicides downwards have all departed from field conditions by applying 'rainfall' at extremely heavy rates and thereby introduced large errors. Relatively insoluble herbicides go into solution slowly; for example, a sphere of trichloroacetic acid 5μ in radius might dissolve in seconds, whereas a sphere of simazine of identical size would take 30 hr. Therefore, unless rainfall is prolonged, the more insoluble substances may resist leaching due to their failure to go into solution, although sufficient water *in toto* may be applied to take all herbicides into solution.

Upward movement of a herbicide in the soil is induced by the evaporation of the soil water. Evaporation will take place almost entirely from the surface of a wet soil, but as the capillary conduction system breaks down the rate of loss of water falls sharply. This upward movement of herbicides will accentuate a tendency for herbicides to be concentrated near the surface of the soil in dry weather.

Even at a rate of application of 100 gal./acre the sorption of a spray solution into the capillaries of a dry soil lump can be quite pronounced and subsequent release of the chemical from such places is by the slow process of diffusion. The only way to counter this appears to be spray when the soil is wet. The development of granular herbicides promises to overcome some of these difficulties since the amount of rainfall necessary to extract the herbicide from the granule is probably sufficient to ensure thorough wetting of the easily accessible sites in the soil before the granules release their active content. A simple experiment using a freely soluble dye added to plaster-of-paris columns showed that the extraction of the dye passing through the columns was far more complete and far quicker if the columns were first wetted with water.

Dr. W. van der Zweep (Wageningen) contributed to the meeting a consideration of the practical use of herbicides in relation to their persistence in the soil. Frequently, the rate of application and the chemical used are determined by the persistence that can be tolerated by the following crops. In certain cases control and not eradication of weeds is a penalty that must be accepted if a following crop is to be safeguarded. Mechanical incorporation of a chemical into the soil increases the persistence if the chemical has a high vapour pressure. In other cases incorporation improves the effectiveness of the chemical and

also assists subsequent breakdown so that a following crop may be sown with increased safety. Persistence may be varied according to the formulation of the chemical used. Dr. van der Zweep brought his contribution to an end by appealing for information on the half-life of herbicides, a half-life determined in the first instance in standardized laboratory tests. Such a system could be extended to field studies where the variation would be apparent and could be measured.

The full proceedings of the meeting together with reports of the discussion which followed each paper will be published by Blackwell Scientific Publications, Ltd., later this year.

COLLOID AND POLYMER SCIENCE

CONTINUING earlier seminars[1] in the Department of Chemistry of University College, London, nine lectures were given during February 25–March 18. Drs. C. H. Bamford and J. A. Kitchener summarized work (some of which has been published[2,3]) on calculation of free-radical reactivity and stability of colloidal model suspensions. Prof. J. A. V. Butler discussed some flow properties of dilute solutions of deoxyribonucleic acid in 0·2 *N* sodium chloride, the rate of shear being varied in the range 0·5–30,000 sec.$^{-1}$. At the high rate of shear no permanent change of the solute occurred, but some disentangling of networks. Using the theories of Kuhn and Kuhn[4] and Cerf[5], for the results obtained at low rate of shear, it appears that the deoxyribonucleic acid molecule is somewhat flexible. Dr. P. W. Allen described the grafting of methylmethacrylate polymers on natural rubber latex using hydrogen peroxide, together with a polyethylene amine, as initiators. Under these conditions the graft co-polymer is mainly formed on the surface of the latex. If oil-soluble initiators are used, polymerization of methylmethacrylate occurs inside the latex. Dr. C. Robinson reviewed recent work[6] on liquid crystals of synthetic polypeptide and described a new effect: if a concentrated solution of poly-γ-ethyl-L-glutamate is illuminated with white light, brilliant iridescent colours are reflected. This is explained as an optical analogue of an X-ray powder diagram. There are indications that deoxyribonucleic acid solutions can also form liquid-crystals.

Prof. A. Katchalsky discussed the application of the thermodynamics of irreversible processes[7] to the study of colloid–chemical phenomena. The treatment of entropy production as the sum of the products of generalized forces and conjugated flows was outlined. Sufficiently slow flows are linearly dependent on all the forces operative in the system, through a set of phenomenological equations, the coefficients of which obey Onsager's law[8] and constitute a symmetrical matrix. The phenomenological equations provide a convenient formal framework for the description of electrokinetic phenomena[9], and permit the establishment of the classical Saxen relations on a thermodynamic basis. A similar treatment[10] of polyelectrolyte solutions leads to new correlations between transport phenomena, such as diffusion, sedimentation and conductance. The thermodynamic approach is useful in the analysis of membrane permeability[11–13]: contradictions inherent in the conventional equations do not occur and a consistent set of equations is obtained, suitable for the description of the transport of electrolytes and non-electrolytes through membranes[14]. The coefficients of these equations were transcribed in terms of frictional forces and distribution coefficients, which characterize the interaction of the permeant solutes with the membrane. In the last lecture, Prof. Katchalsky discussed some fundamentals of mechanochemistry[15].

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EFFECTIVENESS IN INDUSTRY

THE presidential address of Mr. W. E. Bargett to the Institution of Metallurgists, though specifically directed to that profession, had much of direct interest for other technologies. In discussing the training of both graduates and juniors he pointed out the necessity of realizing that the former, though 'educated', have still to receive a 'training' even if during this period they can give little immediate return for their salaries. Equally, "the inefficient utilization of junior people's time is out of keeping with the demands of industry for greater numbers of well-qualified technologists and must be eliminated through sheer economic necessity". It is a general experience that a bright junior is quickly frustrated when called upon to do repetitive work which provides little experience and makes no particular intellectual demand. The interesting suggestion was made that a solution of this problem might be found by employing older people who may have had no special training, but who have sufficient intelligence to learn all that is required to carry out specific operations, appreciating not only what to do but also why it is done in a certain way. Experience has shown that there is no difficulty in filling

posts of this kind very successfully. They have, indeed, a certain attraction for a particular type of man who is satisfied to continue doing a routine job.

Mr. Bargett, though appreciating the temptation to look for rapid solutions of industrial problems, expressed his strong belief that the effort expended would be more efficiently used in gaining a fuller understanding of the problem itself, that is to say, in finding the best, rather than the quickest, solution. Concluding with a few examples drawn from his own work to illustrate the points made, he mentioned the search for an answer to the urgent problem of overcoming the service-cracking at elevated temperatures of a low carbon steel containing ½ per cent molybdenum. The suggestion that the trouble was due to grain-boundary effects which might be eliminated by the addition of 0·002 per cent of boron proved successful, but also, as a most unexpected by-product, led to the yield-point of the steel being doubled and the tensile strength being raised by 50 per cent. Further work led to the production of a weldable steel with a tensile strength of 70 tons per sq. in. in the air-cooled condition.

HEALTH SERVICES IN THE U.S.S.R.

IN October 1958, twenty-three individuals from twenty-one countries and territories were invited by the World Health Organization to visit the U.S.S.R. to study its health services. Some of the members of the group were public health administrators, others were medical specialists, and all were engaged in medical or health work in their own countries. An extensive itinerary was planned by the Government of the U.S.S.R. in order to allow the group to see as much as possible of the country's health services during their stay of a little over five weeks. On their return, the members of the group prepared a report which has recently been published*.

The health services in the U.S.S.R. are organized to meet the requirements of a specific social system and philosophy, and are designed to provide comprehensive medical care for the whole population through the integration of curative and preventive services at all levels of administration. The basic health philosophy in the U.S.S.R. is one of 'prophylaxis' by health protection, and it places the responsibility for the health of the people on the State. The Soviet Government has recognized the part played by health in the economy and development of the country, and the fact that certain population groups (children, workers, etc.) require specialized medical attention.

The existing health services of the U.S.S.R. are the result of development during a period of forty-years research. The complicated structure evolved in this way consists of a number of institutions and functional units so interlocked that the result is a unified system embracing all health disciplines and reaching into every locality in the fifteen constituent republics, to the smallest and most remote villages and farming areas. The basic principle of this structure is highly centralized planning and supervision, coupled with almost complete executive and operational decentralization, permitting about 90 per cent of problems to be dealt with at local levels, without disturbing the general and basic pattern in any way.

The various units constituting the organizational structure of the health services of the U.S.S.R. may be classified into six groups, each group representing a defined function. The six functional groups are: (1) the directing, planning and supervising group; (2) the academic and scientific group; (3) the advisory group; (4) the educational group; (5) the executive group; (6) the operational group.

The health services of the U.S.S.R., both preventive and curative, are available to the whole population free of charge, regardless of social and economic status. The services of health workers, whether highly trained medical specialists or trained *feldschers*, reach every home even in the most remote and isolated localities. This is possible because of the 'State' character of Soviet medicine, and although private practice is not forbidden, it is so restricted that it exercises no influence whatever on the general set-up of health services in the country. The State character of Soviet medicine is further illustrated by the structure of the health budget for the Union. The health budget for 1958 makes provision for an expenditure of more than 40 billion roubles. This means that approximately 200 roubles will be spent for every member of the population in one year. But this does not represent the total expenditure for health in the Union, for apart from the official budgets, other bodies and institutions are also required to budget for additional expenditure on health.

The collective farms provide the buildings and equipment, and bear most of the cost of maintenance of their own health units and maternity homes; the health authorities are only responsible for the salaries of medical and para-medical staff. The same applies to industrial concerns. Furthermore, only part of the capital cost of buildings comes from the official health budget. Finally, certain medicines prescribed for ambulatory patients have to be paid for by the patients themselves.

The Soviet concept of health has resulted in a realignment of services which appears to be unique. There are three main medical fields in the U.S.S.R.: (*a*) adult medicine, (*b*) child medicine, (*c*) hygiene and sanitation.

Soviet scientists feel quite strongly that a child is not just a miniature adult: it is a different being with its own specific physical and physiological functions, and therefore requires a specific medical approach. Pædiatrics is therefore not a post-graduate speciality of general medicine, but rather a separate study which begins during the under-graduate period. A medical student must choose at the beginning of the third year of his course whether he wants to become a doctor for adults, or a doctor for children, and, depending on the choice, will follow a different line of study.

The third medical field is that of hygiene, which produces graduates known as 'hygienists'. Preventive medicine is divided into (*a*) personal preventive

*World Health Organization. Public Health Papers, No. 3: Health Services in the U.S.S.R.: Report prepared by the Participants in a Study Tour organized by the World Health Organization. Pp. 58. (Geneva: World Health Organization; London: H.M. Stationery Office, 1960.) 2 Swiss francs; 3*s.* 6*d.*; 0.60 dollar.

services, which are integrated into the respective fields of adult and childhood medical studies, and (*b*) non-personal services (including environmental and occupational health), which constitute the major subjects at the undergraduate faculties of hygiene. Hygienists staff the sanitary and epidemiology stations and may further specialize in nutrition, municipal hygiene, and industrial hygiene. It appears that, in many respects, the medical hygienists of the U.S.S.R. fill the posts usually occupied by sanitary engineers in other countries.

Scientific research is a most important function of all medical units in the U.S.S.R. Research is planned and co-ordinated, however, and it includes basic, clinical, applied and operational aspects. The system of special research institutes and advisory councils, together with the hierarchical structure of research units, ensures the proper utilization of funds and directs research towards the more pressing problems of the nation.

All heads of divisions and departments of the medical services are medical graduates. Even those departments which deal with sanitary engineering, nursing and para-medical training are headed by doctors. There is no nursing council as such, and the post of sanitary engineer is non-existent. Engineers are attached to departments dealing with buildings and construction, but their task is that of a constructional engineer rather than of a specialized sanitary engineer. The lay sanitarian or sanitary inspector, as known in other countries, is unknown in the U.S.S.R., and his task is performed by doctors, assisted by *feldschers*. Nor are the posts of social workers and social anthropologists known in the U.S.S.R.

The number of doctors in the U.S.S.R. is high, there being about one doctor to every 570 of the population, and in some regions, such as Georgia, as many as 1 in 400. The ratio of male to female doctors is about 50 : 50, but in some areas 70 per cent of practising doctors are women. The total number of medical graduates in the U.S.S.R. in 1957 was 360,000, a striking increase over the figure for 1913, which was 23,000. It is estimated on the basis of existing facilities for medical education that, by the end of the new 'seven-year' plan in 1965, there will be 490,000 doctors in the country, which will give a ratio of one doctor to every 460 inhabitants.

There were also 99,921 mid-wives working in the U.S.S.R. in 1957, as well as 71,151 *feldscher*-mid-wives and 14,714 *feldschers*. It is estimated that by 1965 there will be 1,470,000 qualified nurses in the country, or 3 qualified nurses to every doctor.

BIOLOGICAL EFFECTS OF ATOMIC RADIATION

IN 1956, a report with this title was published by the U.S. National Academy of Sciences. It contained the findings and recommendations of six committees dealing with genetics, pathology, meteorology, oceanography and fisheries, agriculture and food supplies, and disposal and dispersal of radioactive wastes. During the intervening years these committees have continued to collect information in their separate fields, and they have now published their findings and conclusions in the same form as before*. The report is shorter than the first one, in which much space had to be devoted to explanations of fundamental scientific facts and principles. The presentation is again admirably clear and as free from technicalities as possible; the main points are condensed into a separate, concise and easily understood "Report to the Public". Several appendixes bring more detailed information and calculations; technical reports are in preparation. The following are some of the more important statements.

At present, delayed fall-out from the stratospheric reservoir is the major source of airborne artificial radioactivity and will remain so unless large-scale testing is resumed. There is a non-uniform distribution of strontium-90 in fall-out, with a maximum at lat. 40–50° N. Fall-out is greatest in spring and is mostly brought down by precipitation. The half-residence time of radioactive debris in the troposphere is about 2–4 weeks; in the stratosphere it depends on the latitude at which the debris was injected. For injections in equatorial regions, the half-residence time is 2–5 years; for injections in temperate and polar zones it is less than 1 year. If there are no further additions to the atmospheric burden, most of the remaining radioactivity in the atmosphere will have settled down by 1962–63; the ground concentration will then be about twice that of November 1958. It is certain that all living things, man included, now have a radioactive body burden higher than in the pre-atomic era, and although the present levels in foodstuffs are low, the cumulative and retained isotope burden in man must be considered.

The estimate of genetical radiation damage has gone up as a result of the recent increase in fall-out and the previously unconsidered contribution to it of carbon-14. On the other hand, the finding that in spermatogonia and oocytes—but not in mature spermatozoa—of mice and *Drosophila* a given dose of radiation produces fewer mutations when administered at low than at high intensity may mean that previous estimates of genetical radiation damage have erred on the high side. In the absence of more information on the effects of dose-rate and dose-fractionation, the committee continues to recommend that for the general population the average gonadal dose accumulated during the first 30 years of life should not exceed 10 r. of man-made radiation, and should be kept as far below this as practicable. In human tissue cultures, doses of 25 r. produce detectable chromosome breakage in a significant proportion of cells. A slight, but statistically significant, deviation from the normal sex-ratio was observed among children of survivors of the Japanese atom bomb explosion; possibly this might be due to the action of sex-linked mutations affecting prenatal viability.

In animals, a shortening of life-span through radiation has been observed not only for the irradiated individuals themselves, but also for their progeny. In man, shortening of life has not been demonstrated after small doses, but is expected to occur after substantial doses of whole-body irradiation. Part of

* National Academy of Sciences–National Research Council. The Biological Effects of Atomic Radiation: Summary Reports from a Study by the National Academy of Sciences. Pp. xi+90. A Report to the Public on the Biological Effects of Atomic Radiation based on the 1960 Summary Reports of the National Academy of Sciences Committee on the Biological Effects of Atomic Radiation. Pp. iii+19. (Washington, D.C.: National Academy of Sciences–National Research Council, 1960.)

this expected decrease in life-span will be due to leukæmia, which is known to affect over-exposed individuals with more than average frequency. The question whether diagnostic X-radiation during pregnancy increases the incidence of leukæmia in the children cannot be decided on the available evidence. Neither can it be decided whether there is a lower threshold to the production of tumours and leukæmia by irradiation, but the sub-committee dealing with hæmatological effects thinks it prudent to assume that for leukæmia no threshold exists. Mammalian embryos are readily affected by low doses of ionizing radiation. One of the most sensitive known indicators of chronic biological damage is the gradual reduction in number, motility and viability of sperm, resulting from small chronic radiation over-exposure. In dogs, effects on sperm have been observed at chronic dose-rates of only ten times the maximum permissible value for human occupational exposure.

Considerable space is devoted to recommendations for future research, especially in the field of genetics. The need for basic research is stressed, and the relevance of mutation research on all forms of life, including bacteria and viruses, is pointed out. On the other hand, attention is directed to problems that arise in human populations and have no counterpart in animal societies. Thus: "human society depends upon a diversity of performance among its members and on very high mental qualities among a few of them. A human civilization might collapse by becoming qualitatively inadequate, even though reproductive selection of certain kinds were acting at high intensity and the number of individuals in the population remained at a level that was previously optimal". These sentences have an obvious relevance to the questions discussed in the leading article "Evolution of Mankind" in *Nature* of February 13.

C. AUERBACH

MODERNIZATION OF THE STOCKHOLM CYCLOTRON

IN two papers published during 1952, H. Atterling and G. Lindström described the initial performance of the fixed frequency 225-cm. cyclotron at the Nobel Institute of Physics, Stockholm (*Arkiv for Fysik*, **4**, 559; 1952, and *Nature*, **169**, 432; 1952). A more detailed description of the machine, which has operated reliably and stably for several years producing internal beams of protons, deuterons, alpha-particles and heavy ions (carbon, nitrogen, oxygen and neon ions), is given in two recent articles in *Arkiv for Fysik* (**15**, 483 and 531; 1959). The oscillator-frequency used recently is 8·1 Mc./s. and with this frequency the machine accelerates ions to a nominal energy of about 11 MeV. per nucleon at a radius of 90 cm. Internal beam currents of approximately 300 μamp. of 22-MeV. deuterons are obtained.

In March 1958, the cyclotron was shut down and taken to pieces for remodelling. Previously it had been run exclusively with internal beams, but when the dees were designed provision was made for the subsequent mounting of an internal d.c.-deflector in one of the dees and during the reassembly of the dees the deflector is being installed.

The machine is housed in an underground hall, resting directly on rock and surrounded on all sides by rock, clay and earth. On the top it is shielded by a water pond two metres deep. The control room is in a three-storey building above ground, and the connexion between the building and the cyclotron hall is via a stairway or a lift to the bottom of a shaft from which a corridor leads to the hall. A removable 150-cm. concrete thick wall partly closes the corridor, leaving an opening 140 cm. broad which can be closed by a concrete door weighing 35 tons. The first article contains full details together with drawings and photographs of the building; the magnet; the radiofrequency tank and dee systems; and the ion-source of the cyclotron. In the second article the techniques used in the work on heavy-ion acceleration in the cyclotron together with some results of the investigation of the energy-distribution for $^{12}C^{4+}$ ions are outlined. It is shown that the distribution contains a low-energy component in addition to the higher-energy peak of the ions. Other applications of the accelerated heavy-ions, in particular for the production of transuranium elements, are briefly mentioned.

DAILY FOOD CONSUMPTION BY SOUTH AFRICAN UNIVERSITY STUDENTS

IT has been the practice of the Department of Physiology of the University of the Witwatersrand to request students, as part of their physiological training, to keep a daily record of their food consumption over a defined period of time. The records from one year's class form the basis of a preliminary report by Sonia E. Walker; but it is hoped that data accumulating over several years will afterwards be analysed, and will give some indication of the dietary trends in this student section of the community (*South African J. Med. Sci.*, **24**, December, 1959).

The food consumption-levels of the students investigated are similar to those reported from other parts of the world. In a survey of dietary levels of households in the United States, the average consumption per person per day was found to be 2,760–3,260 calories, 87–115 gm. protein and 133–161 gm. fat. The share of total calories derived from carbohydrate was 43 per cent, from protein 13 per cent, and, from fat, 44 per cent. A more recent report based on the estimation of apparent civilian consumption *per capita* per day in the United States gives mean daily intakes of 3,160 cal., 96 gm. protein, 378 gm. carbohydrate and 144 gm. fat. In the United Kingdom a similar estimation of food consumption per head of civilian population per day gave 2,890 cal., 380 gm. carbohydrate, 112 gm. fat, and 90 gm. protein.

The mean daily fat intake of the male group of South African students was 110 gm. This is considerably less than the average American intake, but similar to the daily intake in Britain. The mean protein consumption of the male medical students is 102 gm. per day, which is higher than that found in the United States or Britain.

For practical purposes, the majority of the medical students could probably be classed as sedentary workers, as a large part of their day is spent in sitting in lectures and in dissecting rooms, or in doing laboratory work. Recommended daily allowances for young males doing sedentary work range from 2,400 cal. and 70 gm. protein according to American standards to 2,250 cal. and 66 gm. protein according to British standards.

The present study showed a marked sex difference in food intakes between the male and female students. This is in agreement with observations made on college men and women in the United States, where the men were found to consume more meat, milk and cereals than the women students.

The calorie intake of the Johannesburg women students was slightly less than that found in a group of American students of comparable age, body-weight and height.

DISTRIBUTION OF ATMOSPHERIC OZONE; A PRELIMINARY ANALYSIS OF SOME INTERNATIONAL GEOPHYSICAL YEAR OBSERVATIONS

By J. MacDOWALL

Royal Society International Geophysical Year Expedition, c/o The Royal Society, Burlington House, London, W.1

THE three years of total atmospheric ozone observations at Halley Bay (75° 31′ S., 26° 37′ W.) do not fit into the world-wide picture of the ozone distribution first measured by Dobson[1] from 68° N. to 44° S. Maximum amounts of ozone are not observed until the equivalent of 2–3 months after the seasonal maximum in high northern latitudes, and the highest values observed in the southern hemisphere are lower than in the north.

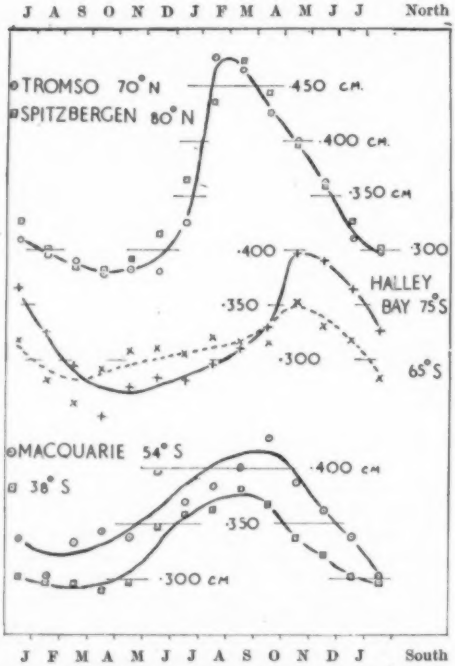

Fig. 1. Shows that the type of seasonal variation of total ozone at Halley Bay is also discernible at Argentine Islands (65° S.), and the seasonal variation at both stations differs greatly from the variation found at high-latitude northern stations

Fig. 1 shows that the type of seasonal variation at Halley Bay is also present, but much less definite, ten degrees to the north at Argentine Islands; the seasonal variation of ozone at both stations differs greatly from the variation found at high-latitude northern stations. International Geophysical Year observations of total ozone at Macquarie Island, Melbourne, and Brisbane show a more 'normal' seasonal variation.

The International Geophysical Year observations from twelve northern and five southern stations are used in Fig. 2 to illustrate the marked difference

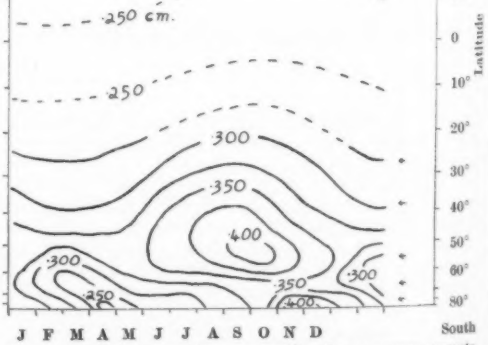

Fig. 2. Smoothed monthly mean results of ozone measurements from seventeen stations illustrating the marked difference between the hemispheres in the seasonal variation of the total quantity of atmospheric ozone

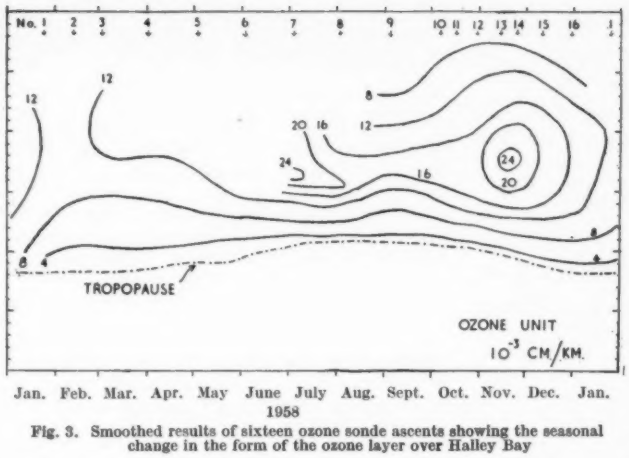

Fig. 3. Smoothed results of sixteen ozone sonde ascents showing the seasonal change in the form of the ozone layer over Halley Bay

between the hemispheres in the distribution of atmospheric ozone at latitudes higher than 55°. The small arrows on the right of the diagram indicate the latitude of the seventeen stations; ozone values for low latitudes, shown by dotted lines, are chiefly due to the work of Normand[2]. There is a close similarity between the hemispheres in low latitudes, but south of 55° S. completely new features of the ozone distribution are revealed, particularly during July–September (compare with the ozone distribution during January–March in the north). In these early spring months the amount of ozone in the north increases very rapidly with latitude, but in the south least ozone is found at high latitudes.

Upper air conditions are well known to be related to the total amount of ozone[3]. Short period fluctuations of 3-day mean values about the 10-day running mean of air temperature and pressure are related to short-period fluctuations of ozone at Halley Bay, but they are less closely related than in south-east England. However, at Halley Bay, 10-day running mean of air temperature and pressure are found to bear little or no relation to the total amount of ozone, whereas in south-east England these measurements are closely related.

During 1958 a series of sixteen chemical ozone sondes were flown from Halley Bay; the sonde was designed by Dr. A. W. Brewer[4] at the Clarendon Laboratory, Oxford, to fit on a standard Kew type radio sonde as used by the Meteorological Office, London. The results of these flights are used in Fig. 3 to draw a smoothed height/time cross-section of ozone concentration over Halley Bay. Apart from the intrinsic interest of these soundings it is of importance to notice at the moment how they confirm the lateness (compared with high northern latitudes) of the ozone maximum at Halley Bay. The great success in the operation of this somewhat temperamental instrument was largely due to the skill of Mr. J. A. Smith, who joined the Meteorological Group at Halley Bay for 1958.

I take this opportunity to thank my colleagues at the Royal Society Base for their co-operation which made the ozone work successful, Mr. J. Farman of the Falkland Island Dependencies Survey, for provisional International Geophysical Year daily values of total ozone at the Argentine Islands, and Dr. G. M. B. Dobson for the great benefit of his advice.

This work formed a part of the programme of the British National Committee for the International Geophysical Year; a full discussion of the results will be published in due course[5,6].

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[6] MacDowall, J., in "The Royal Society I.G.Y. Antarctic Expedition, Halley Bay, Coats Land, Falkland Island Dependencies, 1955–1959", 3 (1961).

THEORY OF AURORAL STRUCTURES

By E. R. HARRISON*
Atomic Energy Research Establishment, Harwell

THE varied structures of the visual auroræ present problems in many branches of physics. In this discussion the subject is simplified to some extent by ignoring so far as possible such questions as where the auroral streams come from, how they gain their energy, and what are the excitation mechanisms. On the other hand, the present treatment collects together some of the properties, such as stability, peculiar to beams guided by the geomagnetic field prior to their entry into the denser regions of the atmosphere. It turns out that stable beams are possible provided they are thin in cross-section. The break-up of arcs into rayed structures can, it appears, be explained by the conditions governing the growth of electrostatic oscillations in the streams. It seems reasonable that quiescent arcs and rays are equilibrium structures, free at least from any gross forms of instability, whereas many of the transient effects of auroral phenomena are the result of unstable streams.

Auroral displays[1] often begin with quiet, homogeneous arcs extending distances of several thousand kilometres along parallels of geomagnetic latitude. A striking feature of many of the arcs is their thinness; Elvey[2] reports that on one occasion an arc had an estimated thickness of 0·25 km. Frequently several arcs are close together and as many as six have been observed in a distance of 25 km. along a geomagnetic meridian. Later in the display the arcs tend to brighten and then disrupt into rayed structures. Later still, the display may change to pulsating

* Temporarily on leave at CERN, Geneva.

surfaces and flaming auroræ. Apart from the amorphous and relatively transient forms of auroræ, the two basic types of structure in any display are the arcs and the rays[3]. They occur either independently or together in various combinations. Both types extend upwards along the geomagnetic lines of force. Arcs extend vertically for approximately 50 km. and seldom more than 100 km. Rays, however, can have heights of several hundred kilometres, and also diameters of less than 0·5 km. Doppler shifted hydrogen lines are observed in homogeneous arcs[4] (but so far not in rays) and indicate maximum incoming proton velocities of at least 3×10^8 cm.sec.$^{-1}$. In the arcs it seems that the excitation is produced partly by protons, whereas in rays the excitation is primarily by electrons[5].

Commencing with the idea that the beams responsible for producing the auroræ may consist of streaming plasma, one finds that in the absence of electric fields the electrons are rapidly eliminated and the plasma is converted into an ion beam. Let H_0 be the geomagnetic field strength where the plasma is injected at an initial temperature T_0. With no collisions, the magnetic moments are invariant, and the mean transverse kinetic energy increases as kT_0H/H_0 at the expense of the longitudinal kinetic energy, as the particles approach the Earth. In a streaming plasma the electrons have a relatively small kinetic energy and they are therefore brought to rest more quickly than the ions. Thus, if kT_0 is of the order of 10 eV., and protons have initially an energy of 10^8 eV., the electrons are arrested when $H \simeq 5H_0$, and the protons when $H \simeq 10^4\, H_0$. Assuming that injection occurs at a distance of 10 Earth radii, the plasma is converted into a proton beam at 6 Earth radii, and at the Earth's surface the protons (assuming no collisions) have lost 10^4 eV. When, however, the plasma is density-modulated into bunches, either by spatial or time growing oscillations (see below), the associated electric fields do not have to be large to trap and drag along some of the electrons and increase their transverse kinetic energy at the expense of the energy of the protons. This mechanism may account for some of the energetic electrons found in the auroral zones[6] and in the outer Van Allen belt[7], particularly if the initial transverse energy is larger than that assumed above.

The beams producing the quiescent arcs and rays are evidently guided in a rigid manner by the geomagnetic lines of force. An analysis of the problem shows that there is lateral stability when the Maxwell stress tensors of the geomagnetic field exceed the stress tensors of the self-fields and of the beam itself[8]. Neglecting for the moment the self-fields, this requires that:

$$H^2/4\pi > n_b{}^+ m_b{}^+ v^2 \qquad (1)$$

where $n_b{}^+$ is the number density and $m_b{}^+$ the mass of the ions in the beam, and v is the velocity component parallel to the geomagnetic field H. When the 'tension' of the geomagnetic lines of force is greater than the 'negative tension' of the momentum flux, as given by (1), the beam is hydromagnetically stable. The upper atmospheric density is comparable with or greater than the beam density, and when (1) is not satisfied the Alfvén phase velocity has complex values and the beam is unstable against transverse perturbations[9].

From (1) it follows that the stress tensors of the self-magnetic field components are small compared with $H^2/4\pi$ provided the beams which produce the rays have a radius d, and the beams which produce the arcs a semi-thickness d, given by:

$$d < \delta_b \qquad (2)$$

where $\delta_b = (m_b{}^+ c^2/4\pi n_b{}^+ e^2)^{1/2}$ is the collision-free penetration depth of an electromagnetic field for the beam ions. In the case of protons $\delta_b = 2 \times 10^3 n_b{}^{-1/2}$ km.

Condition (2) is also necessary if the Larmor radius of the beam ions in the transverse self-magnetic field is to exceed the beam radius. This has been shown elsewhere[10] for cylindrical beams in terms of Budker's parameter $\nu = (d/2\delta_b)^2$, and it seems that for non-relativistic speeds (2) is necessary for a streaming type motion, whereas $d > \delta_b$ applies for plasmas in which the relatively small drift motions are maintained by electric fields. For constant-speed ions the ratio d/δ_b varies at most by $\sqrt{2}$ in any beam following the geomagnetic flux lines; thus if (2) is true at one point, it is also approximately true for all points of a constant speed beam. In spite of both (1) and (2) instabilities may still occur, particularly in arcs, due to transverse force fields producing drift motions[11]. For example, it has been suggested that drift motions are responsible for the folding of arcs into draperies[12].

Using the plasma parameter β, defined as the ratio of the thermal and magnetic pressures, (1) is equivalent to $\beta < 2kT/m_b{}^+ v^2$. Beams with energy sufficient to penetrate only to a magnetic field strength H are initially stable if $\beta < H_0/H$, provided the magnetic moments are invariant. One notices that the beam ions have a mean Larmor radius in the geomagnetic field of $\delta_b\beta^{1/2}$.

We first consider the possibility that the incoming proton beams proceed directly from a large-scale source or stream external to the geomagnetic field. If a stream of particles is incident on regions of the geomagnetic field of strength H_0, and we assume that the convergent flux lines are parallel to the stream velocity, then by Busch's theorem[13] it is found that if (1) is satisfied, then (2) is also satisfied. In one particular model one imagines that tongues of plasma from the solar wind are injected into the unstable outer surface of the geomagnetic field[14]. One might tentatively extend such a model by suggesting that the outer Van Allen belt contains the particle injections which have failed, due to beam instabilities, to produce an immediate auroral effect; such instabilities could be responsible for the energetic electrons found in the belt. It has been suggested[15] as an alternative possibility that auroral beams originate from regions within the Earth's magnetic field, as, for example, the outer Van Allen belt mentioned above. The difficulty with this suggestion is that there is no evidence[7] of the trapped energetic protons at distances of several Earth radii which are at least necessary for the quiet arcs.

So far what has been said applies more or less equally for the beams which produce both the ray and arc type structures. However, the possibilities are strong that the situation is different when one turns to the subject of electrostatic oscillations in beams passing through a plasma. Because of the long-range Coulomb forces, initial density fluctuations under certain conditions can grow into bunches, and the coherent interactions lead to a rapid loss of momentum from the beam[16]. In the present treatment it is sufficient to consider only those coherent interactions in the beam which occur high above the

auroral display. These interactions will determine whether the beam survives to contribute to the auroral display, and whether the beam will produce an arc or a ray. At lower altitudes, approaching the ionosphere, the electron density rises to a value which makes it extremely unlikely that any non-relativistic beam of electrons or protons larger in radius than a few metres is stable. As a result the energy of the incident streams is converted into fluctuation and drift energy of the atmospheric electrons. Radio studies of the aurorae[17] have shown for some time that there are electron density fluctuations having in some cases the form of bunches elongated along the magnetic field. These fluctuations have been attributed principally to atmospheric turbulence. In the present discussion it is suggested that the coherent bunching observed in the ionospheric and auroral regions are possibly the result of incoming particle streams.

From the electromagnetic equations, the equations of motion and continuity, we have:

$$\nabla_\perp^2 E_z + k_z^2 \varepsilon E_z = 0 \qquad (3)$$

$$1 - \frac{\omega_b^2}{(\omega - k_z v)^2} - \frac{(\omega_a^+)^2 + (\omega_a^-)^2}{\omega^2} = \varepsilon$$

where E_z is the longitudinal component of the electric field, $k = (k_z^2 + k_\perp^2)^{1/2}$ is the wave number, and ω_b and ω_a are the plasma frequencies for the beam and plasma, respectively. It is assumed that only longitudinal oscillations occur. Interactions between the beam and the stationary atmospheric electrons is complicated by the fact that there is momentum transfer and the moving electrons in turn interact with the atmospheric ions. Comparing the rates of momentum transfer (Ohkawa, T., privately communicated) one finds by an approximate treatment that the electrons are dragged along at a speed of vn_b/n_a, which is of the order of 10^6 cm.sec.$^{-1}$. But this method neglects Landau damping due to the thermal motion of the electrons and the velocity spread of the beam. It has been shown[16] that an electron stream interacts with stationary ions when it has a velocity exceeding its mean random speed, or in this case when $vn_b/n_a > (2kT/m^-)^{1/2}$. This requires an electron thermal energy of less than 10^{-3} eV. The thermal energy is certainly greater and at high altitudes might be as large as 10 eV., and in this case beam interactions with the electrons can be neglected.

Consider first the case of short transverse wave-lengths, given by $k_\perp d > 1$. Using the dispersion equation obtained from (3) one finds that there are spatially growing waves (k_z is complex) for $k_z < k_\perp$, and time growing waves (ω is complex), when:

$$k_\perp < \frac{1}{v}\{\omega_b^{2/3} + (\omega_a^+)^{2/3}\}^{1/3} \qquad (4)$$

and there is no velocity spread in the beam. At distances of several Earth radii the atmospheric ion density is possibly of the order 10^2 cm.$^{-3}$, and the beam density is, say, of the order 1 cm.$^{-3}$, and therefore $\omega_a^+ > \omega_b$. It follows from (4) and the assumption that $k_\perp d > 1$ that a sufficient condition that there are no instabilities is:

$$d < \frac{v}{c}\,\delta_a^+ \qquad (5)$$

where $\delta_a^+ = c/\omega_a^+$. For $v/c \sim 10^{-2}$ the beams must have a semi-thickness or radius of $d < 0{\cdot}2$ km. When this condition is not fulfilled the beam loses its momentum in a distance:

$$L \simeq \frac{\lambda}{2\pi}\left(\frac{\omega_a}{\omega_b^+}\right)^{2/3} \ln\Lambda \qquad (6)$$

where λ is the wave-length of the disturbance, and the logarithmic term has a value between 10 and 100 and depends partly on the mean square fluctuation $[(\Delta n/n)^2]$. The effect of a large velocity spread[18] in the beam is difficult to assess without some knowledge of the distribution function. The growth-rate of instabilities will be reduced and possibly there will be a relaxation in the stability condition (5).

Condition (5) is sufficient for stability when $k_\perp d > 1$. If we regard the transverse standing waves as dividing the beam into raylets of diameter $k_\perp d = 1$, then (5) is the necessary condition for stability in each raylet. At low altitudes the electron interaction cannot be neglected and the stability condition becomes:

$$d < \frac{v}{c}\,\delta_a^- \qquad (7)$$

and for $n_a^- \sim 10^6$, this gives $d < 5$ cm. Thus, practically all beams lose energy due to coherent interaction as they approach the ionosphere.

For long wave-lengths the electric fields are no longer confined to the beam and the appropriate boundary conditions must be used for solving (3). As a result, the frequencies are reduced by $(k_z^2 d/k_y)^{1/2}$ for sheet beams which produce arcs when $k_y d < 1$ (where k_y is in the transverse direction along the geomagnetic longitude), and approximately by $k_\perp d$ for cylindrical beams which produce rays when $k_\perp d < 1$. The conditions that there are no time and space growing waves, and therefore the beams are stable, are:

$$\text{(arc)} \qquad d < \frac{v}{c}\,\delta_a^+ (k_y d)^{1/2}$$

$$\text{(ray)} \qquad d < \frac{v}{c}\,\delta_a^+ \left\{\frac{2}{\ln(1/k_z d)}\right\}^{1/2} \qquad (8a, b)$$

For ray beams (8b) is sensibly the same as (6) with the exception of the logarithmic dependence on $1/k_z d$. Arc beams, however, are always unstable for sufficiently long transverse wave-lengths. Thus, when an arc beam becomes unstable the transverse standing waves grow rapidly, and for short transverse wave-lengths for which $k_y d \to 1$ the stability is eventually determined by the ray condition (8b).

There are, therefore, a number of elementary conditions necessary for the stability of auroral beams which are broadly covered by (1), (2), (5) and (8a, b). Presumably, in the early stages of an auroral display the beams have a low density and there is no serious growth of coherent interaction at high altitudes. A diffuse source ejecting particles towards the Earth will produce thin homogeneous arcs extending along the geomagnetic latitudes. During the auroral display the beam density apparently increases and the arc beams become unstable; there is then a loss of beam energy. Transverse density variations in the arc beams, however, can lead to the formation of ray beams, which are more stable. According to this treatment the arc is the more fundamental of the two types of structure, and the presence of rays must indicate that arc type beams are arrested in regions possibly high above the auroral display. In these regions there are considerable fluctuations which modulate the down-coming rays, and at lower levels these fluctuations grow spatially, as in a travelling wave amplifier, due to an increase in the density and a reduction in the temperature of the atmospheric

electrons. The oscillations in the ray beams may account for their luminous length, the loss of proton energy due to the atmospheric electron drag is possibly a reason why no hydrogen lines are observed, and the increased energy of the electrons[10] which are excited or dragged down by the beams may also explain the electron impact excitation which predominates in ray structures.

Acknowledgments are gratefully made to Tihiro Ohkawa for a number of informative discussions on plasma and beam oscillations, and to Lloyd Smith for discussions and comments on the treatment outlined in this paper.

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INFRA-RED SPECTRA OF AMINO-ACIDS AND RELATED SUBSTANCES IN WATER

By Prof. FRANK S. PARKER and Prof. DONALD M. KIRSCHENBAUM
Department of Biochemistry, State University of New York, Downstate Medical Center, Brooklyn, New York

ADVANCES both in infra-red instrumentation and in the availability of barium fluoride windows for the manufacture of infra-red cells to contain aqueous solutions stimulated us to undertake a systematic investigation of infra-red spectra of various small molecules of biological importance, including amino-acids and related amines. Until recently, most infra-red spectrophotometric measurements on compounds of biological interest have been made in mulls, in films, in organic solvents, or in potassium bromide pellets[1]. However, following Coblentz's[2] early work with water, Gore *et al.*[3], Blout[4], and others have used aqueous solutions for infra-red studies of certain materials. The present article contains some of the results of our work on amino-acids and derivatives.

Infra-red spectra were recorded as described in a previous publication[5]. Concentrations are recorded in the legends to the figures.

Fig. 1 shows the spectrum of water between 950 and 1,600 cm.$^{-1}$, and typical examples of solubility effects in the aliphatic amino-acids. It is seen that saturated solutions of glycine and alanine have distinctive spectra. The solubility of glycine[6] is 25 gm. per 100 gm. of water at 25°; the solubility of L-alanine is 16·5 gm. per 100 gm. of water at 25°. These amino-acids still show distinctive spectra at 5 per cent concentrations in water; thus glycine still shows distinctive absorption bands at 1,404, 1,319 and 1,124 cm.$^{-1}$; and alanine at 5 per cent concentration still shows the distinctive bands at 1,408, 1,351, 1,299, 1,212 and 1,105 cm.$^{-1}$, among others.

Fig. 2 shows, however, that saturated solutions of valine, leucine and *iso*leucine have very few distinctive absorption bands, as one would expect from their solubilities. The solubility of DL-valine is 7·1 gm. per 100 gm. of water at 25°; the solubility of L-leucine is only 2·2 gm., and the solubility of L-*iso*leucine is only 4·1 gm.

Fig. 3 shows the spectra of proline, serine, threonine and hydroxyproline. The effects of changing the structure from a primary hydroxy compound, serine, to a secondary hydroxy compound, threonine, can be seen. Zeiss and Tsutsui[7] have shown that in alcohols with a saturated chain, a band appears between 1,075 and 1,010 cm.$^{-1}$ for primary alcohols. On the other hand, in secondary alcohols the band

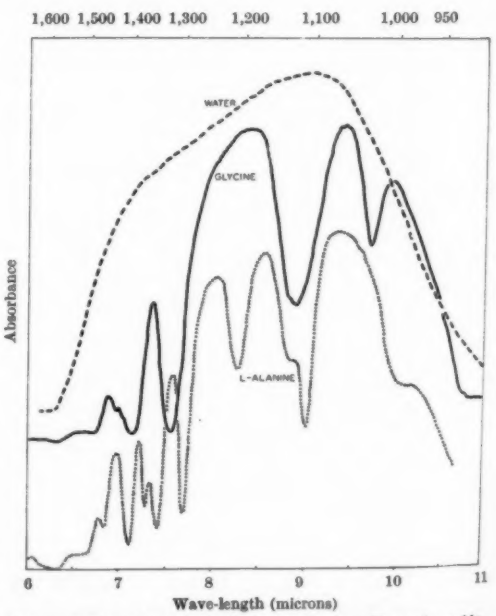

Fig. 1. Spectra of water, glycine and L-alanine. The amino-acids were in saturated solutions. Barium fluoride cell, 0·05 mm. thickness

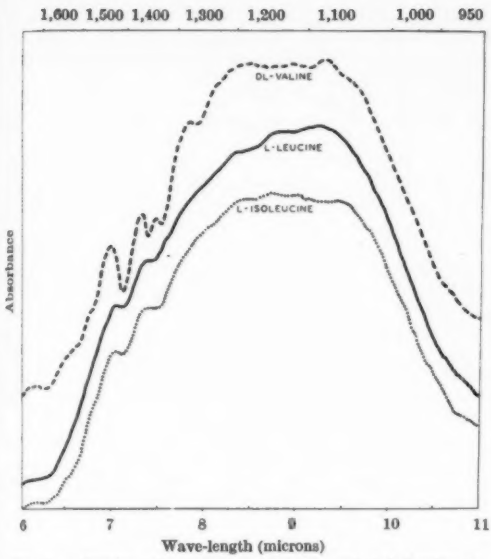

Fig. 2. Spectra of DL-valine, L-leucine and L-*iso*leucine in saturated solutions

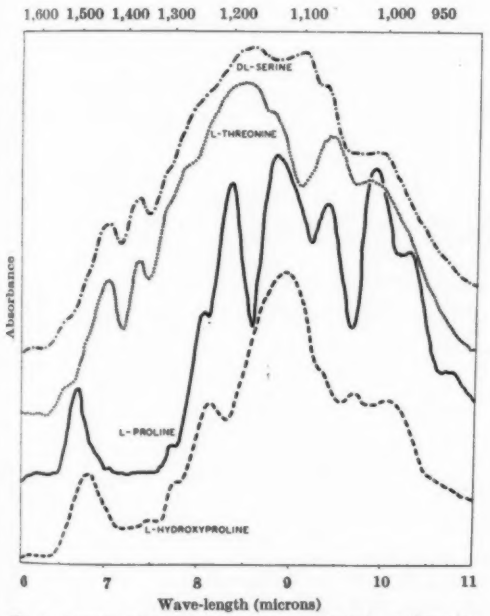

Fig. 3. Spectra of DL-serine (5 per cent solution); L-threonine (5 per cent solution); L-proline (saturated solution); and L-hydroxyproline (saturated solution)

appears between 1,119 and 1,105 cm.$^{-1}$. It is of interest, therefore, that serine has an absorption band at 1,020 cm.$^{-1}$, which is in the range for primary alcohols; and threonine absorbs at 1,100 cm.$^{-1}$, which is in the range for secondary alcohols. However, hydroxyproline, which contains a secondary —OH group on a cyclic imino-acid, does not show absorption where one would expect a secondary alcohol to absorb, namely, from 1,105 to 1,119 cm.$^{-1}$; rather, there is a band at 1,205 cm.$^{-1}$.

It is of interest to note the distinctive differences between the spectra of the imino-acids proline and hydroxyproline, particularly the band in proline at 1,163 cm.$^{-1}$. These differences can perhaps be used to advantage in the examination of the conversion of proline to hydroxyproline, an important process in the biosynthesis of collagen[8]. Although saturated solutions of proline and of hydroxyproline were used, 5 per cent concentrations of these amino-acids can still be differentiated.

Aspartic acid is known to undergo decarboxylation of either the α- or the ω-carboxyl group to form

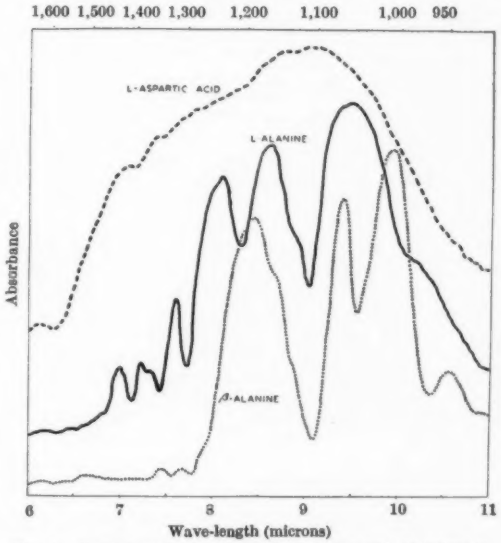

Fig. 4. Spectra of L-aspartic acid, L-alanine and β-alanine in saturated solutions

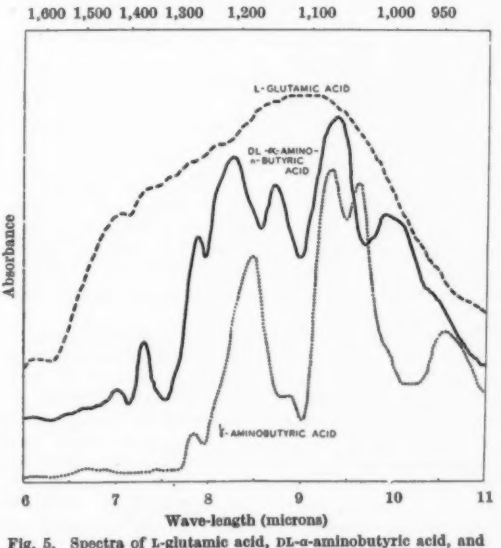

Fig. 5. Spectra of L-glutamic acid, DL-α-aminobutyric acid, and γ-aminobutyric acid in saturated solutions

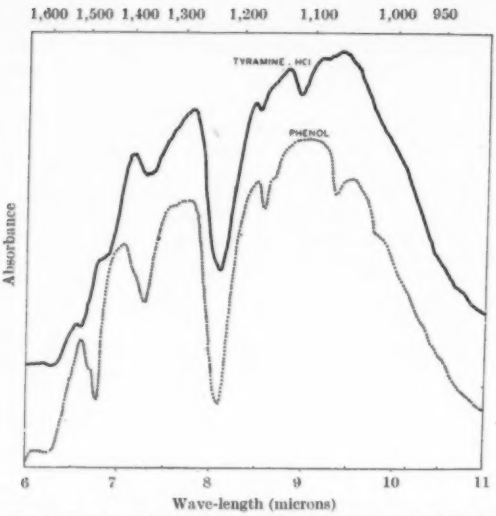

Fig. 6. Spectra of tyramine hydrochloride (10 per cent solution) and phenol (7·5 per cent solution)

either β-alanine or alanine. Examination of Fig. 4 shows that it may be possible to follow such changes using infra-red techniques. There are distinct differences between the spectra of aspartic acid, L-alanine and β-alanine. Infra-red techniques would thus permit one to deduce which end of aspartic acid has lost its carboxyl group. Similarly, in Fig. 5 are shown the spectra of L-glutamic acid, DL-α-aminobutyric acid and γ-aminobutyric acid. Either of the aminobutyric acids can be obtained by decarboxylation of glutamic acid. It is thus possible to distinguish between glutamic acid and either of these decarboxylation products by their infra-red spectra.

With the aromatic and heterocyclic amino-acids, the problem of low solubility of the dipolar ionic form again appears and this renders the demonstration of distinctive spectra difficult. However, the decarboxylation products display distinctive spectra; for example, tyramine (Fig. 6), which is the decarboxylation product of tyrosine, displays a distinctive spectrum as the hydrochloride. Comparison of this spectrum with that of phenol shows that the main absorbing group in tyramine between 950 and 1,600 cm.$^{-1}$ is the phenolic moiety. Tyrosine was too insoluble to study.

The absorption characteristics of many other amino-acids, related homologues and decarboxylation products are being investigated in our laboratory and will be reported elsewhere[9].

A preliminary report of this work was presented before the American Society of Biological Chemists in Atlantic City, New Jersey (*Fed. Proc.*, **18**, 299; 1959).

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A HYPOTHESIS OF CONTRACTION IN STRIATED MUSCLE

By Dr. M. SPENCER and Dr. C. R. WORTHINGTON

Medical Research Council Biophysics Research Unit, Wheatstone Laboratory, King's College, London

WE wish to suggest a mechanism by which the system of sliding filaments in striated muscle, proposed by H. E. Huxley and Hanson[1] and by A. F. Huxley and Niedergerke[2], might be caused to exert a contractile force during excitation. The mechanism is probably also applicable to those smooth muscles which have a double array of filaments[3]. It relies on electrostatic forces between sites on the actin and myosin filaments, and does not necessarily require mechanical linkage between them. It does not involve contraction or folding of the filaments, and thus differs from previous models using electrostatic forces[4].

The mechanism was suggested by the results of recent work by one of us[5] on X-ray diffraction from striated muscle fibres. The results showed that, in addition to orders of the previously observed axial spacing of 410 Å. attributed to actin, orders of an axial spacing of 435 Å. could be seen which were attributable to myosin. The arguments on which this assignment is based have been presented elsewhere[5]. The spacings are significantly different, and can be observed together in the same X-ray diffraction pattern, and in both the wet and the dry state. Work on living muscle is in progress in this Laboratory.

Basic Assumptions

Let us assume that excitation of a striated muscle is ultimately transferred to the actin filaments where they meet the *Z*-lines. (There is evidence that excitation is transferred from the surface membrane to structures called triads in the sarcoplasmic reticulum[6]. In frog muscle the triads lie opposite each *Z*-line; in certain other muscles there are two per sarcomere, displaced from the *Z*-line by a quarter of the sarcomere length.) The terminology is illustrated in Fig. 1. Suppose also (in common with Szent-Györgyi[8]) that the actin and myosin filaments are, prior to stimulation, mutually repelling each other. The repulsion is assumed to be between sites on the filaments which carry like electrostatic charges. The sites are assumed to be spaced at 410 and 435 Å. along the actin and myosin filaments respectively. It seems likely that the sites on either the actin or the myosin filaments, or on both, are really projections from them. Electron micrographs of striated muscle show 'cross-bridges'

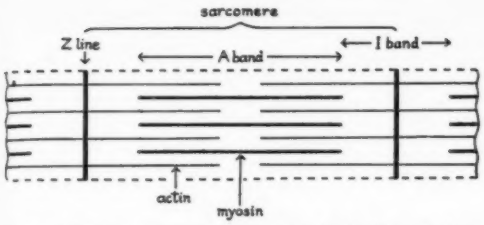

Fig. 1. Diagram of the arrangement of molecular filaments in a striated muscle fibre, according to Hanson and Huxley. During contraction the actin and myosin filaments slide past each other

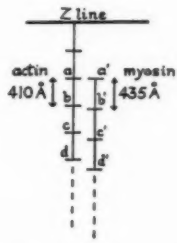

Fig. 2. Diagram of two filaments in a sarcomere, showing proposed sites of electrostatic charge. The difference in spacing has been exaggerated. Sites on the actin filaments are regularly spaced along their length at 410 Å. apart; myosin sites are spaced at 435 Å.

spaced at about 400 Å. which appear to originate from projections on the myosin filaments[7].

Let us now suppose that the excitation transmitted across a *Z*-line sets off a pulse of charge reversal which travels down the actin filaments so that successive sites on each filament are momentarily given an opposite electrostatic charge. (The idea that some change in properties is propagated down the filaments is not new.) The mechanism by which this could happen is, of course, quite hypothetical at present. We believe that it might be a charge transfer of the type envisaged by Szent-Györgyi[8]. A rough parallel may be drawn with the passage of a pulse down an electrical transmission line. Each site carrying a reversed charge would be attracted to whichever sites on the adjacent filaments were nearest to it.

Consequences of the Hypothesis

(*a*) *Free contraction.* Consider the effects on only two filaments, one of actin and one of myosin, of a pulse of charge-reversal travelling down the actin filament (Fig. 2), when the myosin is free to move relative to the actin. Assume, for the sake of argument, that the first charged site a' of the myosin lies initially in register with a site a on the actin. When the charge on a is reversed, there will be no axial force on the myosin filament. However, when the charge-reversal is transferred to b there will be an axial force, upwards in the diagram, on site b'. This would also be true for reversal at sites c and d. If the myosin filament was prevented from responding to the upward force, the charge-reversal at sites farther down the actin would give an opposite force. Since, however, the myosin is free to move, reversal of charge on b will cause movement of the myosin until b and b' are in register. The distance between c and c' is then the same as that originally separating b from b'. If the movement is repeated at each transfer of charge, there will be a continuous upward movement of the myosin. If a and a' are not initially in register, the first movement may be downwards, but all subsequent movements will be upwards. This movement in one direction only may be compared with that occurring in a unidirectional synchronous electric motor.

(*b*) *Contraction under load.* We do not suggest that, except possibly under conditions of zero load, the contraction of muscle involves the synchronous motion just described. The speed of contraction of a muscle varies with its load, and to explain this with a synchronous mechanism one would need to postulate that the speed of propagation of the charge-reversal was controlled by the rate of contraction. However, a study of the actual arrangement of filaments in striated muscle shows that there is no need to invoke such a process, and we shall proceed on the assumption that the speed of propagation is constant.

We know from structural studies[1,2,7] that each myosin filament in a striated fibre is overlapped by actin filaments at both ends. Consider what happens when pulses travel simultaneously from two successive *Z*-lines in a myofibril. We assume that the speed of propagation of the impulse to the beginning of each actin filament is much greater than that of the pulse of charge-reversal down each filament (we shall examine this assumption later). An exact prediction of the sequence of events is impossible without a knowledge of the electrostatic field at all points between the filaments. The analysis which follows describes one possible sequence. It is based on the assumption that, if the pulse arrives simultaneously at a and z (Fig. 3*a*), the myosin filament will adjust its position so that the distance a–a' is the same as z–z'. There will, in the example shown, be a net force pulling the two actin filaments together. Assume, however, that there is 'slip' so that the position is reached later on where the attracting sites on the actin filaments are midway between pairs of sites on the myosin. Sites c and x in Fig. 3*a* illustrate this condition. If the myosin filament remained stationary while the pulses moved on to sites d and w, there would afterwards be a force pushing the actin filaments apart again. However, when the pulses are at c and x, the equilibrium of the myosin filament will be an unstable one; the filament will tend to jump into one of two positions so that c and x on the actin are in register either with b' and x' (Fig. 3*b*) or with c' and y' (Fig. 3*c*). Subsequent transfer of the pulses to

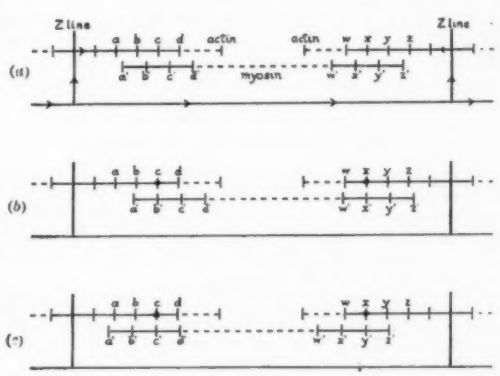

Fig. 3. Three stages in the contraction of a possible model. For explanation see text

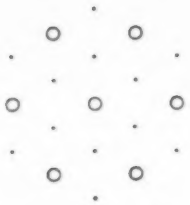

Fig. 4. Arrangement of molecular filaments in a cross-section of the *A* band of a typical striated muscle, as deduced from electron microscopy (ref. 7). Actin filaments are represented by dots and myosin filaments by larger circles

d and *w* will then give rise to forces which still favour drawing together of the actin filaments. We thus see that, provided the myosin is free to make the 'jump' referred to, almost every step in the propagation of the pulses down the actin filaments favours drawing together of the actin filaments. Furthermore, the process is independent of the rate of slip, so that the muscle would exert a tension at all speeds of contraction.

As well as in the special case described above, it can in general be shown that the 'jump' of the myosin filament is essential to the theory; if there was only a steady relative motion of actin and myosin, there would be no net force. However, there is reason to believe that each myosin filament is, in fact, free to move independently. Cross-sections of striated muscle in the *A*-band[7] show that each myosin filament is surrounded by six actin filaments, and is isolated from adjacent myosin filaments (Fig. 4). On the other hand, the fact that every actin filament is adjacent to three others could ensure that the six actin sites which might act on every myosin site were always in register.

(*c*) *Effect of multiple filaments.* It is clear from the above description that an individual set of filaments will exert a tension which varies with time. It will at certain instants be zero and, under certain conditions, it may even be negative for part of the cycle. However, a large number of filaments in parallel will, if their jumps are spread out in time, exert an even tension. There is insufficient evidence to decide whether or not all the filaments in a given sarcomere will act in unison. It is not necessary to assume that all the myosin filaments in a sarcomere are identical. A variation among the myosin periods in a typical muscle of only $\pm$ 0·5 per cent would spread out in time the jumps of different myosin filaments. The variation of $\pm$ 0·5 per cent is less than the experimental error of 1 per cent in measuring the average myosin spacing from a diffraction pattern.

General Properties of the System

A detailed consideration of the expected length-tension and force-velocity curves[10,11] will not be given now. We may, however, note the following properties of the system; some of them are possessed by other possible schemes as well, and have been discussed by previous authors.

(*a*) The maximum tension which the muscle can develop depends on the number of molecular filaments in any one sarcomere, that is, it depends only on the total cross-section of the muscle.

(*b*) The maximum velocity of contraction of the muscle varies as the number of sarcomeres along its length. There is evidence[2,9] that muscles intended for rapid movement often have closely spaced sarcomeres.

(*c*) The isometric tension developed will fall as the muscle is stretched beyond its normal length, because the number of myosin sites overlapped by actin sites will decrease. This means that the number of site interactions per second will also fall (for a given rate of stimulation), and the average force will decrease. A reduction in length will also lead to a fall in tension, for two reasons: first, displacement of fluid by the sliding filaments may increase their separation, and therefore reduce the attraction between sites; secondly, reduction in length will ultimately produce sarcomeres in which the actin filaments have run into each other[7]; this will again result in a drop in the number of interacting sites, and a fall in the tension.

(*d*) The application of an increasing load to the system will lead to an increasing amount of 'slip' from the synchronous motion described, until it just balances the contractile force. It follows that the force-velocity curve must show a decrease in velocity with increasing load.

Thus, qualitatively at least, the expected behaviour of the model is in accord with observation. We have constructed an electromagnetic model which, though crude in that it has only two myosin filaments, illustrates the type of behaviour we have described. It uses coaxial coils to represent the ring of actin filaments around each myosin filament; the latter is represented by a tube containing spaced soft-iron cores. The coils are energized in turn to simulate the passage of the pulse of charge-reversal down the actin filaments.

Discussion

A number of calculations may be made to test whether the assumptions made in the theory are consistent with observations *in vivo*. Examine first the assumption that the passage of the pulse of charge-reversal down each filament takes place at a speed much less than that of the exciting impulse. One way to test this is to compare observed and calculated values for the maximum rate of contraction of a muscle. One would expect that, if our scheme were correct, the maximum rate of contraction of a muscle would correspond with the synchronous motion mentioned earlier. If the velocity of the charge-reversal is v cm./sec., the maximum rate of contraction per sarcomere can be shown to be about $0{\cdot}12\,v$ cm./sec. In a typical muscle of length 1 cm., having sarcomeres of length 2·5μ, the maximum rate of contraction of the whole muscle will then be $500\,v$ cm./sec. Taking an observed maximum velocity of 2 cm./sec. per cm. length of frog sartorius muscle at 0° C. (ref. 12), the value of v must be about 0·004 cm./sec.—much less than the observed velocity of propagation of the action potential along a fibre, which in many cases exceeds 100 cm./sec. (ref. 13). The assumption that there was this large difference in velocities has not therefore led to a prediction which conflicts with experience. We may note here that the average interval between charge transfers from one site to another will be about 10^{-3} sec.

We may also test whether the calculated value of velocity down the filaments is consistent with the observed frequencies of excitation of muscles *in vivo*. One would expect that, for maximum output from a muscle, the frequency of excitation would be such

that one impulse arrived just as the previous pulse of charge-reversal reached the end of the actin filaments. If the frequency was higher than this, the superposition of two pulses on the same set of filaments might lead as often to cancellation of their effects as to reinforcement. (It is of interest to note here that the rate of repetition is in some cases observed to fall when maximum output is not required[12].) If the actin filaments had lengths of 1μ, the time taken for a pulse to travel down them at 0·004 cm./sec. (the velocity calculated above) would be 0·025 sec. The maximum frequency at 0° C would then be 40 impulses/sec. This value is of the right order of magnitude. Calculations based on results obtained at higher temperatures give a higher frequency.

We do not suggest that the nerve impulse transmitted into each *z*-line supplies the energy needed to bring about contraction. We believe that the impulse must trigger a series of reactions which convert chemical energy, perhaps stored in adenosine triphosphate, into electrostatic energy. The theory offers no direct evidence on the reactions involved, and therefore provides no estimate of the heat liberated during contraction.

An important question is whether the theoretical electrostatic attraction between sites can account for the observed tensions developed by living muscle. A typical maximum tension for frog sartorius muscle[10] is 2 kgm./cm.^{2} cross-section. This means that the average force exerted by each myosin filament must be about 3×10^{-5} dyne. If this force is shared between six sites, each of which is opposite an actin site, the average force at each site must be about 5×10^{-6} dyne. The effective force between a myosin site and an actin site is the axial component of the attractive force between them. The component will be zero when the sites are in register. At a large axial separation the component will approach the value of the total force but, due to Debye-Hückel screening, it will be small. Between these two extremes it will rise to a maximum in a way which cannot be predicted without knowing the exact distribution of charges and the ionic conditions in the intervening fluid. In addition to this, there may be large deviations from the simple Debye-Hückel theory under very intense fields. We can, however, make certain assumptions and calculate the maximum component of attractive force between point charges at a given separation.

The most favourable calculation possible is made by neglecting Debye-Hückel screening and assuming a dielectric constant of unity; there are grounds for believing that the relevant dielectric constant may not be as high as that of water. Two sites carrying unit electronic charge would then exert a force of 6×10^{-6} dyne (the average force required) at a separation of about 20 Å. If there were (say) ten electronic charges per site, the separation would be 200 Å. The least favourable calculation is made by assuming the dielectric constant to be that of water, and assuming the Debye-Hückel theory[14] to apply at all separations. The ionic strength, which determines the magnitude of the Debye-Hückel screening, has to be given an arbitrary value; we assume it to be 0·15, a value close to that of human blood serum. The 'thickness' of the ionic double layer[14] will then be about 8 Å. The calculated separation at which unit electronic charges exert the required force on each other is then about 2 Å. This value is, however, so small that the assumptions on which it is calculated cannot be valid, and it is clear that the scheme could not work under these conditions. If, however, there were ten charges per site, the required separation of charges would be 15 Å. This is more of the right order of magnitude, though still somewhat low. For the scheme to be correct we would expect the forces to have appreciable magnitude at distances greater than 25 Å.

It is clear from these calculations that the scheme cannot at present be ruled out on theoretical grounds alone. We may also note that an exact calculation would have to consider the dynamic nature of the process; the attractive force at the beginning of each charge transfer might be greater than that calculated for static conditions, due to the finite time required for equilibrium to be established in the surrounding fluid.

There is one important consequence of the theory which we have not so far discussed. It is that, at any instant during contraction, only a very small percentage (perhaps 4 per cent) of the actin and myosin sites are attracting each other. This implies that the proportion of the biochemist's 'actomyosin' present in contracting muscle will be similarly small, since it seems likely that actomyosin is essentially actin combined with myosin by electrostatic forces. On the other hand, inducement of a charge-reversal of all sites on either actin or myosin would probably cause the filaments to draw together so that they were locked in position by interpenetration of their projections. It seems very likely that such an overall charge-reversal is brought about by removal of adenosine triphosphate from the system[9], since muscles in rigor and glycerol-extracted muscles show the rigidity we have described. The plasticizing role of added adenosine triphosphate and pyrophosphate follows from this idea.

One obvious need in verifying the hypothesis is to extend to the present problem the theoretical work already available on electrostatic forces between charged sites in ionic media[15]. There is, equally, a need to test whether the suggested transfer of charge down the actin filaments does in fact take place. The work of Szent-Györgyi and his associates has shown that this type of process may be vital to many biological systems.

We are indebted to Dr. E. M. Deeley for advice during construction of the electromagnetic model. We would also like to thank Dr. J. Lowy, Dr. J. Hanson and Mr. G. F. Elliott for discussions on the results of muscle research; Dr. M. E. Fisher for advice on theoretical matters; and Prof. J. T. Randall and Dr. M. H. F. Wilkins for encouragement and helpful criticism.

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MELANIN GRANULES AND MITOCHONDRIA

By Dr. RUTH V. BAKER
Department of Pharmacology, University of Oxford
M. S. C. BIRBECK
Chester Beatty Research Institute; Institute of Cancer Research: Royal Cancer Hospital, London
Dr. H. BLASCHKO
Department of Pharmacology, University of Oxford
Prof. T. B. FITZPATRICK*
Department of Biochemistry (Radcliffe Infirmary), University of Oxford
AND
Dr. M. SEIJI*
Department of Biochemistry (Radcliffe Infirmary), University of Oxford

ALTHOUGH the chemical structure of the melanin pigment naturally occurring in mammals is not known[1], much information is available on the mechanism of its formation. Our present knowledge is derived from a study of homogenates or particulate fractions prepared from mammalian pigment cells[2] as well as from histochemical observations on intact cells, with both radioactive and non-radioactive pigment precursors[3]. These studies have established that in mammals melanogenesis is the activity of a specialized type of cell, the melanocyte, which is characterized by the presence of a copper-containing oxidase, tyrosinase, a catalyst responsible for the aerobic oxidation of L-tyrosine, the precursor of melanin, to 5,6-dihydroxyindole. The monomeric 5,6-dihydroxyindole then polymerizes to form a compound of large molecular weight[1]. The melanin polymer is believed to be attached, through its quinone linkages, to amino or sulphydryl groups of the protein matrix of the pigment granule.

Localization of tyrosinase in suspensions of cell particles was first reported by Herrman and Boss[4] in preparations of the ciliary body of the bovine eye. In addition to a "specific catalyst for dopa oxidation", these suspensions also contained two enzymes characteristic of mitochondria, succinic dehydrogenase and cytochrome oxidase. In a study of the Harding–Passey mouse melanoma by differential centrifugation, tyrosinase activity was also reported in the particulate fraction, but the structural elements were thought to be "microsomes or particles the size of microsomes which were formerly part of larger aggregates that were dispersed during the experiments"[2].

In 1949, DuBuy, Woods, Burk and Lackey[5,6] reported that preparations of granules isolated from the Harding–Passey mouse melanoma contained, in addition to melanin, cytochrome oxidase, succinic dehydrogenase and cytochrome *C*, and that they also had tyrosinase activity. These workers arrived at the conclusion that the melanized cytoplasmic particles in the mouse melanomas, which in addition to tyrosinase activity also possessed cytochrome oxidase and succinic oxidase and dehydrogenase activities in considerably varying degree, represent one of several modified forms of mitochondria, but generically mitochondria in any event.

In recent years, several authors have studied the morphology of the melanin granules from various sources in electron micrographs[7]. The melanin granules in human hair bulb appear as dense ovoid or rod-shaped bodies, varying in length from 0·4 to 1·0μ, and in breadth from 0·1 to 0·5μ. In the melanocyte there occurs, distal to the nucleus, an area which contains numerous filamentous mitochondria together with many small vesicles. The centre of this area is believed to be the region where the melanin granules are formed. It is free from melanin granules; but in its periphery there occur structures the size of melanin granules made up of concentric shells upon which variable amounts of dense material are formed; these are considered to be early stages of formation of melanin granules. Both Dalton[8], using Cloudman *S*91 melanoma cells, and Birbeck and Barnicot[7], using human hair bulbs, have concluded that melanin granules and mitochondria differ in size and internal structure. Woods[9] maintains that the melanin granules are melanized mitochondria because of "their high specific activity for enzymes characteristic for mitochondria, their oxygen-dependent staining with janus green B, and the inverse relationship between them and the number of amelanotic mitochondria in the same cells". The need for clarification of this problem is emphasized by Dalton[8], who makes a plea for correlating electron micrographs and chemical data on both melanin granules and mitochondria after centrifugation.

Such an investigation has now been carried out and a separation of mitochondria from melanin granules has been accomplished by the density-gradient

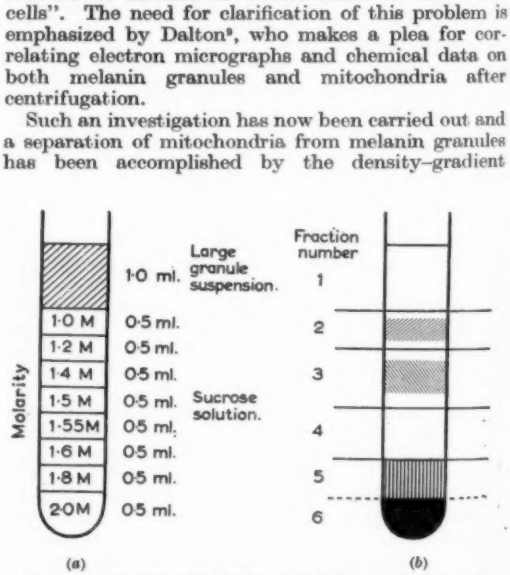

Fig. 1. *a*, Gradient tube before centrifugation; large-granule suspension (in 0·3 *M* sucrose) was added immediately before centrifugation. The gradient was prepared 18 hr. earlier; *b*, gradient tube after ultracentrifugation at 103,000 *g* for 1 hr. The three opaque regions are shown diagrammatically. The places where the tube was cut are also indicated. Fractions numbered 1 to 6

* Present address: Department of Dermatology of the Harvard Medical School at the Massachusetts General Hospital, Boston 14, Massachusetts.

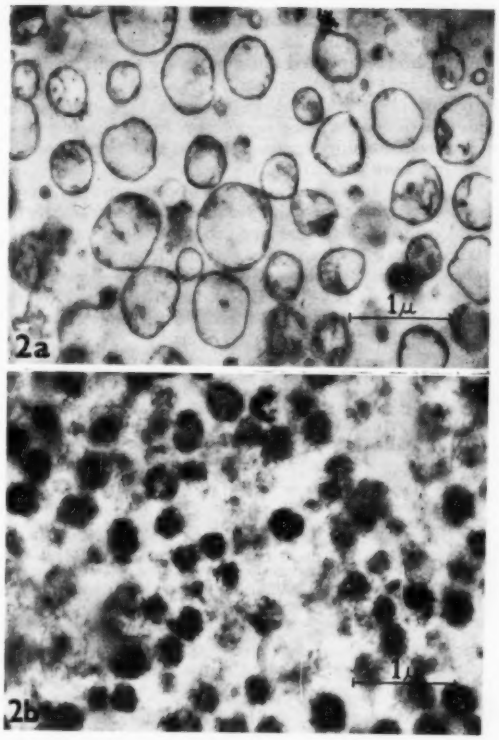

Fig. 2. *a*, Electron micrograph showing elements of fraction 3 (mitochondrial fraction) isolated by gradient density centrifugation. Fraction 3 is a pure preparation of mitochondria quite free of melanin granules. (Sections of fixed embedded fraction; × 16,000.) *b*, electron micrograph showing fraction 6 (pellet after centrifugation at 103,000 *g* for 1 hr.), isolated by gradient density centrifugation. Fraction 6 is a pure preparation of melanin granules. (Section of fixed embedded fraction; × 16,000)

method of centrifugation; the particle fractions thus obtained have been studied by electron microscopy and by biochemical methods. The centrifugation separated two morphologically distinct structures, one of which, the mitochondria, contained succinic oxidase, whereas the other contained the melanin as well as the enzyme tyrosinase.

The 'large-granule' fraction from a Harding–Passey mouse melanoma was isolated by a low-speed centrifugation at 700 *g* for 10 min., followed by a high-speed centrifugation at 11,000 *g* for 10 min. of the low-speed supernatant fluid; the sediment obtained was washed once and suspended in isotonic sucrose solution (0·3 *M*). It was then placed above a density gradient, similar to that used for the separation of mitochondria from amine-carrying granules[10,11]. The gradient used in the present work is shown in Fig. 1*a*. The gradient tubes were centrifuged for 1 hr. at 103,000 *g* in the swinging bucket rotor *SW* 39*L* of the Spinco ultracentrifuge.

The appearance of the tubes at the end of the centrifugation is shown in Fig. 1*b*. Two narrow bands could be seen, one at the top of the gradient (fraction 2) and a second grey turbid band (fraction 3) between this layer and a relatively clear zone below (fraction 4); near the bottom of the tube a black suspension of particles (fraction 5) and a black pellet could be distinguished. The tubes were cut, using the centrifuge tube cutter kindly presented to the Department of Pharmacology, Oxford, by Dr. E. H. J. Schuster some time ago; a cutter, based on this instrument, has recently been described in a review article by de Duve, Berthet and Beaufay[12]. The positions at which the tubes were cut are indicated in Fig. 1*b* by horizontal lines.

Electron micrographs showed that fraction 2 consisted mainly of cell debris; fraction 3 was an almost pure preparation of mitochondria (Fig. 2*a*). Fractions 5 and 6 contained predominantly melanin granules (Fig. 2*b*). It is apparent that in the density-gradient tube the melanin granules had been separated from the mitochondria; the melanin granules had accumulated in a region of much higher density.

We have determined tyrosinase, succinic oxidase and protein nitrogen content of the various fractions as well as of the large-granule fraction from which they were derived. Tyrosinase was estimated using a 10 : 1 mixture of L-tyrosine and L-dopa as substrates (1·77 μmoles)[13], succinic oxidase by a manometric method[14]. Of the total tyrosinase activity, 94 per cent was recovered in fractions 5 and 6. No tyrosinase activity was found in the mitochondrial layer (fraction 3). Of the succinic oxidase activity, 68 per cent was recovered in fraction 3, 7 per cent in fraction 5, and 4 per cent in fraction 6. Total recovery of succinic oxidase was 83 per cent.

The results of this experiment are given in Table 1. They show that fraction 6 (melanin granules) contains twice as much tyrosinase per milligram of protein nitrogen as the large granules, and that fraction 3 (mitochondria) contains seven times as much succinic oxidase per milligram of protein nitrogen as the large-granule fraction (Fig. 3).

These experiments show that in the Harding–Passey melanoma there exist granules that have the typical appearance of mitochondria in the electron micrographs and contain a high concentration of succinic oxidase, a typical mitochondrial enzyme; these granules are found in the gradient tube in a density layer characteristic of all mitochondria studied whatever their source. In addition, the tumours contained denser granules; these had the typical appearance of melanin granules and they contained the bulk of

Table 1. DISTRIBUTION OF TYROSINASE, SUCCINIC OXIDASE AND PROTEIN NITROGEN AFTER CENTRIFUGATION (AT 103,000*g* FOR 1 HR.) OF LARGE-GRANULE SUSPENSIONS OVER DENSITY GRADIENT OF HYPERTONIC SUCROSE

Fraction	Protein nitrogen			Tyrosinase			Succinic oxidase		
	mgm./ml.	Total mgm.	Recovery (per cent)	Specific activity μl. O_2/hr./mgm. N	μl. O_2/hr.	Recovery (per cent)	Specific activity μl. O_2/hr./mgm. N	μl. O_2/hr.	Recovery (per cent)
Large granules	5·2	15·6	100	166·8	2,604	100	270	4,218	100
1	0·64	1·47	9	0	0	0	0	0	0
2	0·72	1·51	10	0	0	0	57	84	2
3	0·88	2·64	17	0	0	0	1,896	2,862	68
4	0·24	0·62	4	184·8	112·2	2·5	168	104	2
5	0·96	3·65	23	186·0	678	26	76	277	7
6	1·92	5·76	37	307·8	1,770	68	28	159	4

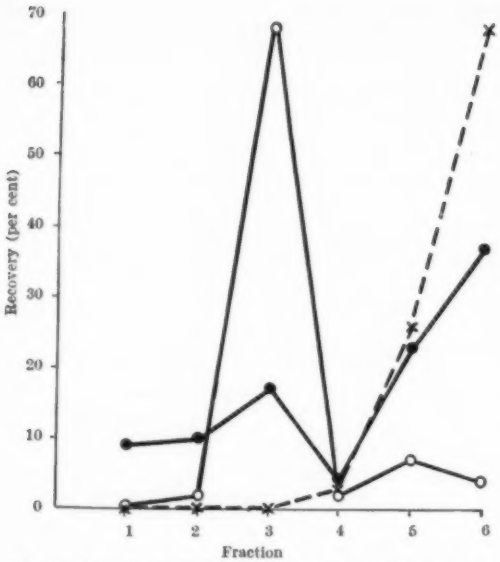

Fig. 3. Contents of fraction 1–6 from density gradient experiment. Percentages are calculated in terms of the amounts recovered in all the fractions. Total recoveries, in terms of contents in large-granule fraction, were: tyrosinase, 96·5 per cent; succinic oxidase, 83 per cent; and protein nitrogen, 100 per cent ×, Tyrosinase; ●, protein nitrogen; ○, succinic oxidase

the tyrosinase activity; they had negligible succinic oxidase activity.

We conclude from our experiments that melanin granules and mitochondria of the Harding–Passey mouse melanoma are distinct cytoplasmic constituents of the melanin-forming cell; each of these constituents is equipped with its own typical enzymes. The method employed does not, by itself, exclude the possibility that melanin granules are derived from mitochondria, but in view of the cytological evidence quoted we see no reason for assuming that such a relationship exists between the two types of structure here described.

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CRITICAL ENERGY BARRIERS IN THE EXCRETION OF SODIUM

By Prof. E. J. CONWAY, F.R.S.
Department of Biochemistry, University College, Dublin

THE secretion of sodium is considered here chiefly with respect to such large-scale secretion of sodium ions as occurs when frogs' sartorii are immersed in Ringer–Conway fluid containing 120 m*M* sodium and then re-immersed for 2 hr. at room temperature in similar fluid containing 104 m*M* sodium and 10 m*M* potassium[1,2]. It was found under such conditions that the re-immersed muscles lost in the average about 20 m.equiv. sodium per kgm. of the muscle before re-immersion, the average concentration after the first soaking being approximately 59 m.equiv./kgm. (ref. 4).

This type of experiment will for convenience be referred to here as the 120/104/10 procedure, and there will be occasion to refer to others such as the 120/120/10, 104/104/10 and the 120/120/30 procedures.

It was found that there was no significant secretion of sodium using the 104/104/10 procedure[3,4], which was in marked contrast to the results of the 120/104/10 procedure. As a tentative hypothesis it was considered that the over-night immersion in fluid with sodium-level of 120 m*M* in some way stimulated the activity of the sodium pump in the subsequent 2-hr. re-immersion period. But it was found also that the 120/120/10 procedure resulted in no significant excretion of sodium, and this was interpreted in the sense that though the pump was here likewise stimulated, there was an entrance of sodium in the re-immersion period which about balanced the excretion. With such procedure the hypothesis was tested by inhibiting the excretion of sodium either by 2 m*M* iodoacetate or by ouabain (10^{-5} *M*) (Conway, Kernan and Zadunaisky, unpublished work), which had been shown[4] to inhibit fully, or practically so, the net secretion of sodium when using the 120/104/10 procedure. With sodium secretion so inhibited it could be expected that sodium would increase within the muscle, in about corresponding amount. But no appreciable increase of sodium was observed at the end of the 2-hr. period.

It was also shown that the explanation did not lie with osmotic pressure differences in the fluids used.

It was then decided to test another hypothesis, namely, that the cause of the marked difference in secretion resulting from the use of the 120/104/10 and the 104/104/10 procedures could arise from the

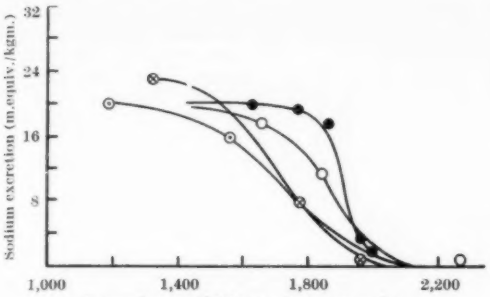

Fig. 1. ●, for series 120/120/10, 120/115/10, 120/104/10, 120/80/10; ○, for series 104/104/10, 104/80/10, 104/104/30; ○, for anaerobic series, 120/120/10, 120/104/10, 120/104/30; ⊗, for 120/120/30, 120/120/60

existence of a critical energy barrier under the conditions. The energy barrier to the secretion of sodium may be written:

$$\frac{dG}{dn} = RT\ln\frac{[Na]_o}{[Na]_i} - E_mF \qquad (1)$$

where n is the number of moles of sodium excreted and $[Na]_i$ and $[Na]_o$ are the concentration of sodium in the fibre water and in the external fluid, E_m being the membrane potential (but the numerical value of $-E_mF$ is positive). If one includes associated movements of potassium or chloride ions, the same equation will result from principles already established[3]. One can lower the energy barrier, therefore, either by reducing the concentration of the external sodium or by increasing the concentration of external potassium, which in turn lessens the membrane potential.

In order to calculate the energy barriers, the membrane potential E_m must be known. Approximate values of E_m were obtained using the Graham and Gerard technique[5] on fibres of the sartorius after about 15–30 min. after the second immersion. Such potential measurements are necessarily inexact for the purpose because of changing conditions. None the less, it is to be expected that they will not be much in error and they are useful data in the present context.

In Fig. 1 the secretion results are plotted against the calculated energy barriers. The occurrence of a critical energy barrier is clearly evident from the graph. This critical energy barrier for the conditions described is of the order of 2,000 calories per equivalent of sodium. But the magnitude of the barrier may be expected to change when the conditions are much different, as mentioned below. Among other things, the graph shows that when the 104/104/10 and 120/120/10 procedures (giving no significant excretion of sodium) are changed to 104/104/30 and 120/120/30 procedures, a large excretion of sodium occurs.

Here it may be noted that when excretion occurs on reducing the external sodium concentration some fall in membrane potential occurs.

Oxygen Uptake as affected by the Critical Barrier and Sodium Excretion

A number of experiments were conducted in which the oxygen uptake was determined by the Warburg technique for the second immersions in the 104/104/10, 120/120/10 and the 120/104/10 procedures. The results are summarized in Table 1. As there was no apparent difference in oxygen uptake or sodium secretion between the 120/120/10 and the 104/104/10 procedures with respect to the secretion of sodium ions or the uptake of oxygen, these experiments were grouped together. Table 1 gives the medians as the most representative values. Fuller details will be published in a paper by Conway, Kernan and Zadunaisky. The number 63 refers to the excretion of sodium as previously established[4], the other numbers to experiments of the present series, each experiment being conducted on three sartorii, and companion muscles being in nearly all cases used for comparison with the 120/104/10 procedure.

It will be seen then from Table 1 that with this series of experiments the 120/104/10 procedure shows a marked excretion of sodium ions, in contrast to the other procedures. (Here the mean value of the excretion is somewhat higher than in the groups previously described.) At the same time, the oxygen uptake is considerably increased throughout this secretion, and the average ratio of the milli-equivalents of sodium excreted to millimoles oxygen gas taken up is 3·84 or close to 4·0. It is to be noted that the increased oxygen uptake here occurs with the same concentration of external potassium.

Further, in a series of 35 experiments carried out with the 120/104/10 procedure, there is, as usual, a rather wide distribution in the amount of sodium secreted in each single experiment. At the same time there is a very significant correlation (0·68 ± 0·1) between the sodium excreted and the oxygen uptake.

Here it may be mentioned that Keynes and Maisel[6] investigated the relation between oxygen uptake and sodium excretion from frogs' sartorii with the muscle sodium somewhat increased by immersion for 30 min. in potassium-free Ringer's fluid. The excretion was measured by isotopic exchange. These measurements at the time appeared quite satisfactory, but it is now known that due to exchange diffusion they can differ widely from the real extrusion[7,4].

Table 1. SODIUM EXCRETION, AEROBIC AND ANAEROBIC, FROM SODIUM-RICH SARTORII

The numbers in brackets refer to the number of experiments (each with three sartorii). The number 63 for the first two results listed refer to experiments from Carey, Conway and Kernan (ref. 4)

Procedure	Sodium excreted (m.equiv./kgm.)		Oxygen uptake (m.moles/kgm.)	
	Means	Medians	Means	Medians
	Aerobic			
104/104/10 }	0·3 ± 0·7	−0·1	10·7 ± 0·5	9·8
120/120/10 }	(63)	(63)	(25)	(25)
120/104/10	22·1 ± 1·6	22·1	16·2 ± 1·6	15·6
	(35)	(35)	(22)	(22)
			Lactic acid formed	
			Means	Medians
	Anaerobic			
120/120/10	−1·1 ± 2·6	−1·3	30·1 ± 2·1	30·1
	(12)	(12)	(12)	(12)
120/104/10	6·4		33·8	
	(4)		(4)	
120/104/30	22·7 ± 2·4	23·1	43·7 ± 2·1	41·6
	(12)	(12)	(12)	(12)

	Mean	Median
Value of the ratio: $\frac{\text{m.equiv. sodium excreted}}{\text{extra oxygen uptake}}$	3·95	3·97
Value of the ratio: $\frac{\text{m.equiv. sodium excreted}}{\text{extra lactic acid}}$	1·75	2·12

(taking differences between the 120/120/10 and the 120/104/30 results)

Anaerobic Secretion of Sodium Ions from Skeletal Muscle

It was shown[3,4] that the excretion of sodium in the re-immersion period of the 120/104/10 procedure could be inhibited in the average by 60 per cent. It was then obvious that, on the average, 40 per cent of the aerobic excretion of sodium could occur anærobically. Similar results were obtained with anoxia. These conclusions were based on 74 experiments as published[4], and were much added to recently.

Such distribution shows a wide scatter, and in an appreciable fraction of the experiments there was very little inhibition, whereas in another it was almost complete.

At an early stage of such work[3], when about 13 experiments had been carried out, in which a high proportion showed very marked inhibition by cyanide, it was thought that the cyanide effect was fully inhibitory, but the accumulation of a large number of further experiments showed that the true average inhibition under our conditions was 60 per cent[5]. Recently, Frazier and Keynes[9] have stated that they were unable to find any cyanide inhibition under their conditions (which were essentially similar to ours, but differing in some points of detail).

It would seem that the concept of the critical energy barrier, and certain recent experiments based thereon, throw light on this difference. It is shown in Table 1 that decreasing the energy barrier by using 30 m*M* potassium ions externally instead of 10 m*M* completely removed all inhibition by cyanide. Thus the difference in the effect of cyanide found by the two laboratories may be referable to different metabolic states in the frog muscle used.

Explanation by the Redox Pump Theory

In this theory (refs. 2, 8, 10, 11) it is supposed that the reductant of a redox system specifically adsorbs sodium ions, and when it loses electrons to a second system these are released into solution. The energy change may be written:

$$\frac{dG}{dn} = F(E_2 - E_1) + RT\ln[Na^+]_i \qquad (2)$$

$F(E_2 - E_1)$ within the cell or fibre is the driving force for the excretion. The manner in which it can be considered to be utilized is as follows. If, instead of existing only in the free fibre water, the system with the adsorbed sodium ion existed in the membrane, transferring its electrons at the outermost edge to another system, with simultaneous release of sodium ions into the external fluid, the energy equation could then be written:

$$\begin{aligned} dG/n &= F(E_2 - E_1) + RT\ln[Na^+]_i \\ &= F(E_2 - E^1{}_1) + RT\ln[Na^+]_e - E_mF \end{aligned} \qquad (3)$$

where $E^1{}_1$ represents a raised potential. From equation (3):

$$F(E_2 - E_1) - F(E_2 - E^1{}_1) = RT\ln\frac{[Na]_e}{[Na]_i} - E_mF \qquad (4)$$

The electrons transferred to the second redox system can either go up the respiratory chain to oxygen or, in the anaerobic state, be finally received by pyruvic or enolpyruvic acid after linking with hydrogen ions to form lactic acid. Now there is a maximum level of $RT\ln[Na]_e/[Na]_i - E_mF$ which is reached when $F(E_2 - E^1{}_1)$ becomes zero following increases of $[Na]_e$ or E_m. If at the outset the value of $RT\ln[Na]_e/[Na]_i - EF$ is greater than $F(E_2 - E_1)$, no electrons can pass through the system and the critical energy-level is exceeded. The magnitude of this critical energy-level can vary with the conditions, and this is provided for in the theory.

Thus, the driving force $F(E_2 - E_1)$ can be expanded:

$$F(E_2 - E_1) = F(E_{20} - E_{10}) - \frac{RT}{F}\ln\left\{\frac{[Red.]_2}{[Ox.]_2} \times \frac{[Ox.]_1}{[Red.]_1}\right\} \qquad (5)$$

where E_{20} and E_{10} are the characteristic potentials of the systems. The expression under the log sign could be expected to vary in different metabolic states of the muscle. We may in this way interpret the fact that in the normal frog *in vivo*, or in very freshly excised muscle, the critical energy barrier must be much higher than 2,000 calories and probably at the level of 4,000 calories. The nearer the muscle to the normal condition, the greater there would seem to be the driving force. This may also explain recoveries (that is, in secretion of sodium and uptake of potassium) under certain experimental conditions when the external potassium is as low as 3·5 m*M* (Harris, private communication).

Thus the facts here mentioned of the critical energy barrier, of the extra oxygen with sodium excretion, and the extra lactic acid with anaerobic excretion, with approximate average ratios near 4·0 and 2·0 respectively as referred to above, are very well explained by the redox pump theory.

In the case of the anaerobic secretion, it may be noted that while there is a very significant relation between the extra lactic acid found and the sodium excreted, there is rather much variability in the ratio mentioned above. One may say that the underlying trend of the ratio is near 2·0. The variability may be explained by somewhat different states of the enzyme systems involved, even using companion muscles. That the raised concentration of external potassium is not responsible for the extra lactic acid, apart from its effect on the energy barrier and the consequent excretion of sodium, would appear from the absence of this effect when comparatively little muscle sodium was present for excretion, as when 10 m*M* potassium chloride was incorporated in the soaking fluid. Also, it may be added, there appeared no contractures at any stage, under the conditions.

Space does not permit of further considerations of important points of detail in this presentation, but it is hoped to deal with these matters in coming communications with Drs. Kernan and Zadunaisky and with Miss Mullaney.

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LETTERS TO THE EDITORS

ASTROPHYSICS

Local Increase of Intensity of Cosmic Radiation

DURING the International Geophysical Year, no important proton event of solar origin was detected on the Earth by the different stations; but on the other hand, high-altitude balloon flights show that arrivals of low-energy protons to the polar regions were rather frequent events. These arrivals of protons are generally preceded by a solar flare associated with a radio outburst of type IV, and are accompanied by ionospheric absorption, total or partial, in the polar regions.

As these events are of considerable importance in the investigation of Earth–Sun relations, we thought it interesting to communicate some local increases of the cosmic-radiation intensity (impact region), detected by stations on the Earth, which seemed difficult to explain at the time they occurred. The correlations between these increases and the information given by other stations or observatories will perhaps make it possible to find the exact cause of these events.

Thus, on December 4, 1957, we recorded at the Paris Cosmic Ray Survey Station (geomagnetic co-ordinates: 51·2° N., 84·7°, altitude: 90 metres), by means of an unshielded telescope detecting the total component, an increase in intensity of cosmic radiation.

Particulars of the equipment are as follows: Thickness of the materials constituting the roof of the Paris Cosmic Survey Station reduced to gm./cm.²: 2·5 gm./cm.². Characteristics of the unshielded telescope detecting the total compound: 3 trays of 7 counters assembled in threefold coincidence. Aperture angle: 130° × 130°. Thickness of the materials which constitute the telescope reduced to gm./cm.²: 9 gm./cm.². Average counting-rate: 227 counts/min. at a pressure of 760 mm. of mercury. Characteristics of the shielded telescope detecting the hard component: 3 trays of 5 counters assembled in threefold coincidence. Aperture angle: 55° × 110°. Shielding: 10 cm. of lead, 1 cm. of steel. μ-meson detection threshold: 165 MeV. Average counting rate: 87 counts/min. at a pressure of 760 mm. of mercury.

The recording of received impulses is done by a printing clock, which marks the times for 10,000 impulses. On average we thus obtain a record covering 44 min. (or 227 counts per min.) for a barometric pressure of 760 mm. mercury.

An increase, of 18 ± 2 per cent total amplitude, the maximum of which occurred at 22 hr. 45 ± 20 min. U.T., began at 21 hr. ± 20 min. U.T. and finished at 0 hr. 15 ± 20 min. U.T. on December 5, 1957 (Fig. 1). It was not detected on the hard component. The effect was therefore preponderant on the soft component.

We inquired whether this event had been recorded by other stations. Only the station at Thule (geomagnetic co-ordinates: 88·0° N., 1·1°) showed an increase of 5 per cent between 18 hr. and 20 hr. U.T. on the nucleonic component[1] (Fig. 1). As the records of this station were only recorded *bi*-hourly, it is possible that the increase would be more prominent on a shorter recording period.

The maximum time between the onset of these two increases of cosmic-ray intensity should be 3 hr. ± 20 min.

This event took place during a period of relatively quiet solar activity. For the whole day of December 4, 1957, only one important radio outburst was detected at Ottawa[2] on 2,800 Mc./sec., at 16 hr. 57·5 min. U.T., coinciding with a flare of importance 1 observed at Huncayo (16 hr. 56 min.–17 hr. 04 min.; max. 16 hr. 58 min. U.T.; —20·5–18° W.)[3].

The delay between this solar event and the increase recorded at the station at Thule should be 2 ± 1 hr.: a delay analogous in rough approximation to those corresponding to the arrival of protons of solar origin[4].

It must be noted that the heliographic longitude of the flare (18° W.) corresponds to the part of the Sun where protons emitted during a flare have a great probability of being observed in the vicinity of the Earth[5]. On the other hand, the increase at Thule took place during a period of small magnetic activity ($Kp = 1^-$ during 18–21 hr. U.T.), whereas the Paris event took place during a period when the magnetic activity began to increase ($Kp = 2^+$ during 21–24 hr. U.T. and 4° during 0–3 hr. U.T.)[6] (see Fig. 1). This may explain the delay noticed between the increases observed on the Thule and Paris records.

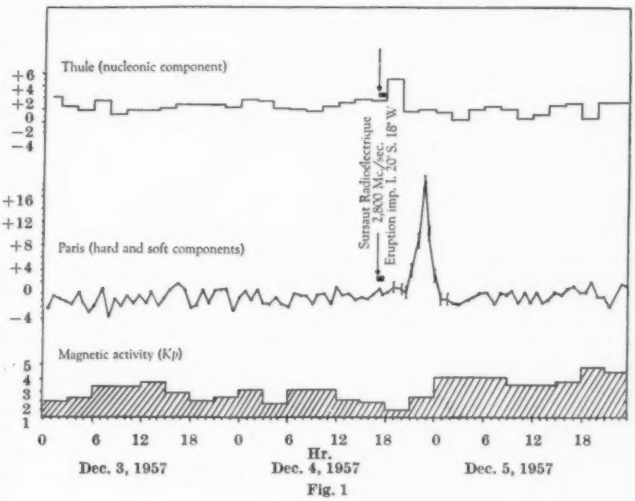

Fig. 1

Indeed, cosmic-ray particles with an energy less than that for geomagnetic cut-off in the latitude of Paris, which are generally forbidden, could in consequence of the increased magnetic activity penetrate by impact at this latitude. An analogous event was recorded at the Paris station on January 21, 1957 (ref. 7).

All these different events and the way in which they occurred suggest an arrival of protons of solar origin; but their small magnitude makes it necessary to have further experimental observations for a definite conclusion.

J.-P. Legrand
A. Helary

Commissariat à l'Energie Atomique,
Limeil-Brevannes, S.-et.-O.

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RADIOPHYSICS

A Highly Directive Rotating Array for 16 Mc./s.

The typical rotating back-scatter sounder[1,2] uses a horizontal 3 or 4 element Yagi for both transmission and reception. The main-lobe width for such aerials is commonly about 60° between half power points, and when the aerial is used in conjunction with a 'transmit–receive' switch the effective lobe width reduces to about 40°. This means that echoes will tend to become 'smeared out' in azimuth to a greater or lesser degree depending on the received signal strength.

One of the investigations being undertaken at Brisbane is concerned with the detection and analysis of echoes from field aligned ionization in the *F* region of the ionosphere[3], and for this purpose it is desirable to achieve some finer degree of discrimination of the azimuthal extent of echoing regions than is possible with the simple Yagi system. Considerable thought has been given to the design of a large low-cost rotatable array of aerials, and some degree of compromise proved to be necessary.

The system chosen consists of 4 three-element Yagis separated by 1·1 wave-lengths, mounted on a truss 228 ft. long pivoted at its centre, and supported by four bogies with normal truck wheels and balloon tyres running on concrete tracks (Fig. 1).

Each Yagi is constructed entirely of aluminium and mounted at the top of a guyed aluminium mast 30 ft. above ground-level. (This height gives maximum signal at an elevation angle of about 25°, a value suitable for the experiments in hand.) All the Yagis are fed in the same phase, and the relative currents are chosen to reduce the intensity of side lobes near the main beam. The computed aerial gain is 150.

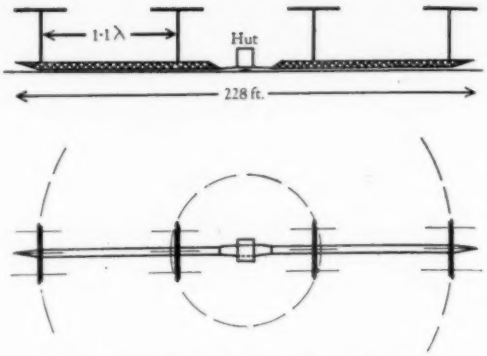

Fig. 1. Elevation and plan views of rotating array

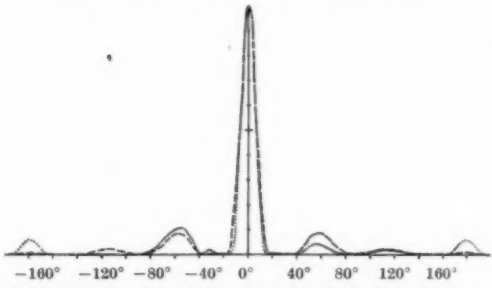

Fig. 2. Radiated power versus array orientation (in the horizontal plane). – – –, Computed power; ...., measured power

A comparison of the computed and measured azimuthal radiation pattern of the aerial array is given in Fig. 2. The two major lobes at ± 55° are reduced from an amplitude equal to that of the central beam only by virtue of the reduced radiation from each individual Yagi in this direction. The high front-to-back ratio of the system is likewise entirely produced by the Yagis. The small degree of asymmetry shown in the measured pattern is not of any

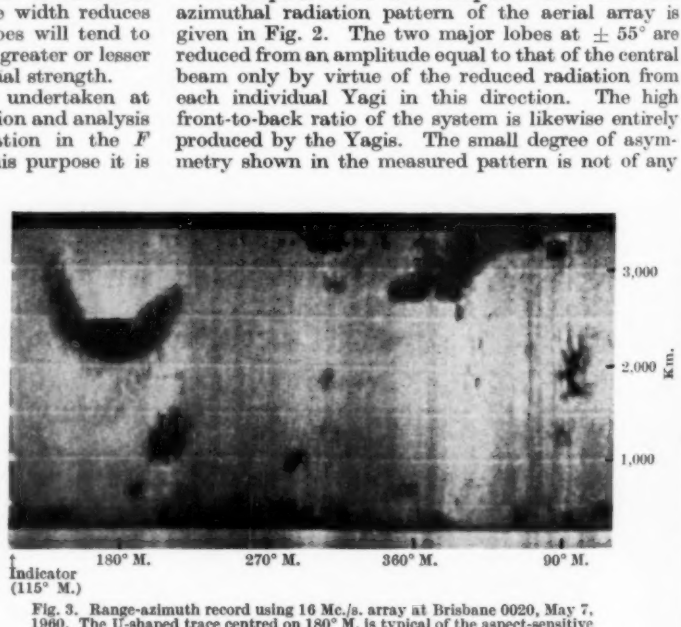

Fig. 3. Range-azimuth record using 16 Mc./s. array at Brisbane 0020, May 7, 1960. The U-shaped trace centred on 180° M. is typical of the aspect-sensitive reflexions obtained

major importance when the array is used both for transmission and reception. Under such conditions the effective beam-width is 8° (to half-power points), and the lobe at − 55° is 19 decibels down with respect to the main beam, thus giving a considerable increase in azimuthal resolution over the use of a single Yagi. The back lobe is 24 decibels down with respect to the forward beam.

The system is rotated by two 1 horse-power electric motors hydraulically coupled and geared down to drive one of the wheels on each outer bogie. Normal speed of rotation is one revolution in 3 min.

Fig. 3 shows an example of a range-azimuth display obtained using the array, which illustrates the particular advantage of the narrow beam for the purpose of identifying aspect-sensitive reflexions.

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J. A. Thomas
R. W. E. McNicol

Physics Department,
University of Queensland,
Brisbane.

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Radar Determination of the Scattering Properties of the Moon

Information about the scattering properties of the Moon's surface has been deduced by Evans[1] from the rapid fading of lunar radio echoes. His results were obtained by picturing the surface as an assembly of randomly spaced scatterers. Such a concept, however, does not take into consideration the distribution in depth of the scattering elements. An approach which does so is that adopted by Feinstein[2], who used Huygens's principle to study the general problem of reflexion from a plane surface having random irregularities that are a function both of the space co-ordinates and of time. With minor modifications to take into account the curvature of the reflecting surface, Feinstein's results may be applied to lunar radio echoes.

Consider first the lunar surface to be corrugated in one dimension only, so that its height h above a mean level can be represented by the random variable $h(x,t)$, where x is distance measured along the surface from its intersection with the line connecting the centres of the Earth and the Moon. If the resultant surface velocity due to the lunar librations is v, and if the co-ordinate plane is determined by this vector and the line of centres, the function h will have the form $h(x - vt)$.

Now assume that h is normally distributed and that the joint distribution of the h's at any two points is bivariate normal. The space correlation function of the signal envelope observed by spaced receivers on the Earth's surface when the Moon is directly overhead can then be shown to be:

$$C(s) = \exp\{ - a[1 - \rho(sR/2D)]\} \qquad (1)$$

where s is separation between receivers, a is $4k^2\overline{h^2}$, k is $2\pi/\lambda$, where λ is the radar wave-length, $\overline{h^2}$ is variance of the distribution of h, ρ is normalized space correlation function of the lunar surface, R is radius of the Moon, D is Earth–Moon distance.

By fitting experimental data to (1), it is possible to determine both the lunar space correlation function ρ and the constant a, since $C(s)$ approaches $\exp(-a)$ asymptotically. It is important to note, however, the occurrence of an approximately 500-fold change in the scale of this function that results from the reflexion of the incident wave-front at the curved lunar surface. Space diversity tests carried out by the U.S. Army Signal Research and Development Laboratory over its circuit from Belmar, N.J., to the University of Illinois, at Urbana, Ill., have verified this expansion in scale.

The autocorrelation function of the received signal envelope at one location on the Earth's surface is given by:

$$P(\tau) = \exp\{ - a[1 - \psi(\tau)]\} \qquad (2)$$

where $\psi(\tau)$ is the autocorrelation function that would be observed at any point on the mean lunar surface that is fixed relative to the co-ordinate system postulated. Because of the relation between x and t, it is immaterial whether we determine $P(\tau)$ or $C(s)$ experimentally, since both contain the same information. Only $C(s)$, however, is expanded by the factor $2D/R$.

The one-dimensional results for the correlation functions are directly applicable to the two-dimensional case, if the variance of h is independent of direction. For the determination of the distribution of angular power, however, the lunar surface must be treated as two-dimensional, and, in addition, the form of the function ρ must be specified. Feinstein[2] has derived the angular distribution for the case where:

$$\rho = \exp(- d^2/l^2) \qquad (3)$$

where d is distance measured in any direction along the lunar surface and l (or more commonly $l/\sqrt{2}$) is called the structure size. Because surface irregularities much smaller than λ will not appear in the reflected wave-front, the smallest value of l determinable from radar observations will be of the order of magnitude of λ. If Feinstein's results are again modified to fit the lunar problem, the angular power distribution is found to be:

$$p(r) = (\exp - a)[\cos(kr^2/R) + \sum_{n=1}^{\infty} \frac{a^n}{n.n!} \exp(- r^2k^2l^2/nR^2)] \qquad (4)$$

In order to determine $p(r)$ completely, we must first assume that $\rho(d)$ has the form given in (3) and obtain numerical values of a and l by fitting observational data to (1) or (2). These values of a and l are then substituted into (4).

Fred B. Daniels

U.S. Army Signal Research and
Development Laboratory,
Fort Monmouth,
New Jersey.

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GEOPHYSICS

Anomalous Leads from a Selected Geological Environment in West-Central New Mexico

A DETAILED study of lead isotopes has been undertaken in order to evaluate variations in the isotopic composition of lead with respect to the geological environment of the sample area. Support for the project is being provided by the New Mexico Bureau of Mines and Mineral Resources division of the New Mexico Institute of Mining and Technology and by the Geophysics Laboratory of the University of Toronto.

A field area was desired in which the number of geological variables would be at a minimum. An area of approximately 5,000 square miles in west-central New Mexico was found to satisfy this requirement. Barite-fluorite-galena deposits are scattered throughout the field area[1,2]. These deposits have identical mineralogy, very similar geological settings with respect to host rock type, and a common depth-temperature type of mineral deposition. Within the field area, the Hansonburg mining district was selected for detailed studies of single galena crystals and for determination of local isotopic detail across parallel north–south mineralized fractures. The other samples have been taken from localities scattered across the field area in order to investigate the possibility of widespread isotopic variations. The location of sample number 1054 is unique in that it is located near deep-seated acid intrusives, but here too the mineralization is very similar to that of the other sample locations[3].

Galena (PbS) was the mineral collected from each site chosen for this preliminary study. Fresh, unweathered specimens were utilized, and wherever possible the entire sample was taken from a single galena crystal. When the samples contained only fine-grained, disseminated galena, the sample was crushed and pulverized to minus 20 mesh, screened, and a heavy mineral concentrate prepared by panning. Fresh galena was then hand picked from the heavy mineral concentrate under a microscope.

Following the sample preparation at the New Mexico Bureau of Mines, the samples were sent to Toronto for analysis. The hand-picked samples of galena were dissolved in hydrochloric acid, and the lead ions precipitated as lead iodide. A Grignard reaction of lead iodide and methylmagnesium bromide at room temperature was used to produce lead tetramethyl. The lead tetramethyl was mass analysed in the Nier-type 180-deg. mass spectrometer operated by the Geophysics Laboratory of the University of Toronto. The precision of the various analyses is shown in Table 1.

Table 1. PRECISION OF ANALYSES

Lead isotope	Sample variation range	Average sample variation
204	$S = \pm 0{\cdot}001$ to $\pm 0{\cdot}011$	$S = \pm 0{\cdot}005$
206	$S = \pm 0{\cdot}02$ to $\pm 0{\cdot}08$	$S = \pm 0{\cdot}04$
207	$S = \pm 0{\cdot}02$ to $\pm 0{\cdot}06$	$S = \pm 0{\cdot}03$
208	$S = \pm 0{\cdot}03$ to $\pm 0{\cdot}09$	$S = \pm 0{\cdot}05$

$S = \pm\left[\frac{\Sigma(x-\bar{x})^2}{n-1}\right]^{1/2}$. This is the precision of an individual mass scan and is not the error of the average values reported in Table 2.

A large crystal of galena from the wall of an open fracture zone in the Hansonburg district was selected for detailed study. The galena crystal, illustrated in Fig. 2, had the form of a cube cut in half along a diagonal plane. The diagonal surface of the crystal was attached to a large breccia fragment of silicified limestone. The crystal was sampled by cutting it into small cubes with a diamond saw. A corner (sample 111) and two samples along a diagonal (samples 222 and 333) were analysed. The positions of these samples are indicated in Fig. 2. The corner sample was found to be distinctly different from the internal samples. The compositions of the crystal samples are listed in Table 2 under sample number 1049.

Samples of galena were collected along east–west lines across the north–south mineralized fractures in the Hansonburg mining district. Small, consistent

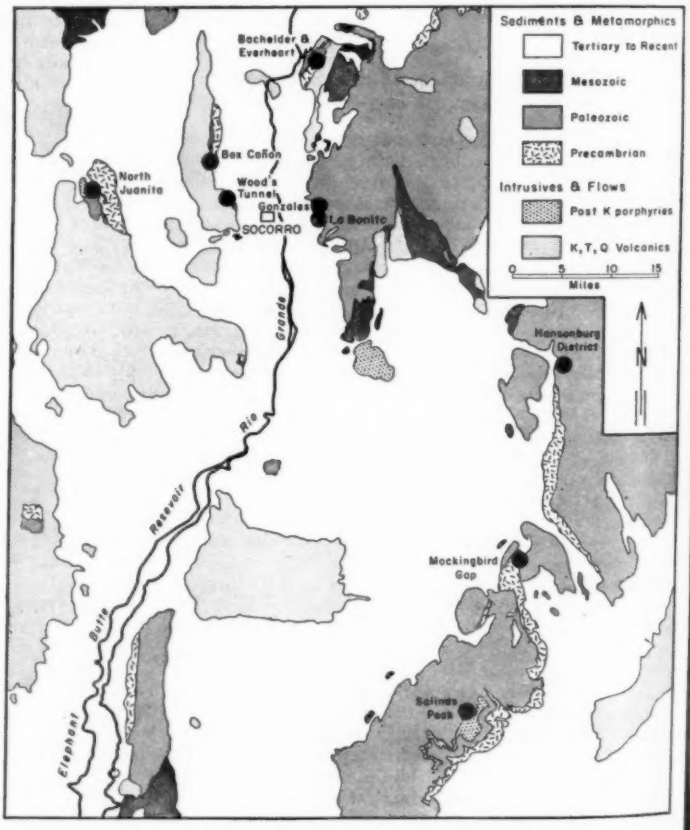

Fig. 1. Map of field area showing location of samples

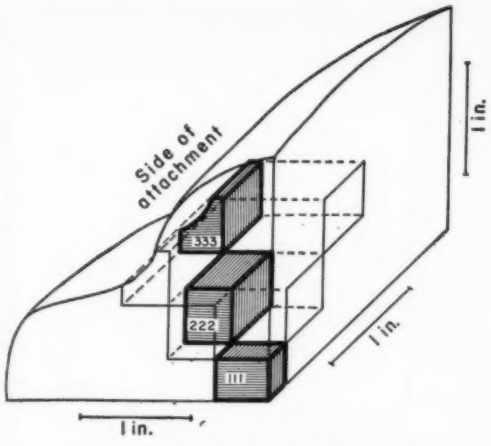

Fig. 2. Single crystal with sample positions shown

variations in isotopic composition were found from west to east. Radiogenic lead was found to increase to the east. Samples 1,040, 1,046 and 1,047, taken from west to east, illustrate this. The sample spacing is 200 ft. between samples 1,040 and 1,046, and 100 ft. between samples 1,046 and 1,047. No north–south trends were noted in the Hansonburg mining district.

Regionally, the geological map of New Mexico[4] suggests a northwest-trending basement structure underlying samples 1,054, 1,059 and 1,065. These three samples have had distinctly less radiogenic lead added to them during their history of emplacement than the other samples taken in the field area. Such isotopic variations suggest that basement structure may be reflected by the isotopic composition of the lead present in the overlying mineral deposits.

When all of the samples are plotted as lead-207/lead-204 versus lead-206/lead-204, they fall in a straight line. The slope of this line, as determined by the least squares method, is 0·0938. Applying the method of Farquhar and Russell[5], the maximum age for the source of the radiogenic lead additions is calculated as 1,530 million years. This figure agrees fairly well with the age of 1,300–1,450 million years given by Tilton and Davis[6] for the basement rocks of the south-western United States.

Table 2. Presentation of Data

Mine name or sample location	Sample number	Percentage			
		Pb-204	Pb-206	Pb-207	Pb-208
Hansonburg district					
Sunshine-Blanchard	1,040	1·252	27·86	20·06	50·82
	1,046	1·247	27·97	20·09	50·68
	1,047	1·242	28·10	19·96	50·70
	1,049 (111)	1·284	27·15	20·47	51·08
	(222)	1·245	27·83	20·06	50·87
	(333)	1·256	27·74	20·14	50·87
Royal Flush	1,041	1·250	27·72	20·12	50·90
	1,042	1·260	27·38	20·21	51·15
Snake Pit	1,043	1·252	27·80	20·04	50·90
Mex-Tex	1,044	1·252	27·76	20·09	50·90
Hurlow	1,045	1·261	27·69	20·18	50·87
Hickey	1,048	1·265	27·60	20·26	50·88
North Juanita	1,054	1·351	25·12	21·20	52·34
Mockingbird Gap	1,059	1·335	25·29	20·96	52·41
Wood's Tunnel	1,065	1·354	25·24	21·22	52·19
La Bonita	1,055	1·173	29·74	19·10	49·99
Gonzales	1,056	1·242	27·83	19·96	50·96
Box Cañon	1,057	1·290	26·66	20·47	51·58
Bachelder-Everheart	1,058	1·263	26·77	20·10	51·87
Salinas Peak	1,060	1·208	28·47	19·46	50·86
	1,061	1·212	28·38	19·48	50·92
	1,062	1·203	28·49	19·43	50·88
	1,063	1·203	28·54	19·37	50·89
	1,064	1·205	28·51	19·45	50·83

The preliminary work reported herein indicates that continued research is warranted within the limited field area selected. The lines of isotopic research being continued by us are: (1) Delineation of basement structure. (2) Single-crystal detail to obtain relative ages for isotopic variations within local areas. (3) Delineation of local isotopic trends, such as the east–west trend found in the Hansonburg mining district.

William F. Slawson

Geophysics Laboratory,
Department of Physics,
University of Toronto.

Carl F. Austin

New Mexico Bureau of
Mines and Mineral Resources,
a division of the New Mexico
Institute of Mining and
Technology.

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PHYSICS

A Method for the Routine Measurement of Particle Shape Factors in the Sieve Range

Size distribution cannot alone describe a particle population, and for some purposes it becomes necessary to measure the particle shape within closely sized ranges. Some instances of the use of shape factors are in estimating approximate surface areas, calculating the number of particles in a size range and investigating the variation of particle shape with size. Since these applications are not confined to one field or industry, interest in shape factor is widespread.

If k and f are the volume and the surface shape factors respectively, then by definition:

$$V = kd^3$$

and

$$S = fd^2$$

where V is particle volume, S is surface area and d is some measure of the particle diameter. It is usual to use the statistical diameter[1–3], but for the present purpose the geometrical mean between the limiting and retaining screen apertures on the British Standard $\sqrt{2}$ scale[4] is convenient. Heywood[5] has given relationships between statistical and sieve aperture diameters for various particle shapes. Clearly, these definitions must be interpreted statistically; V and S represent the mean of a large number of measurements. Consequently, although particle shape factors as defined here are simple in conception, their experimental determination is laborious since it may be necessary to sample, count and weigh several thousand

closely sized particles. However, particularly careful work of this nature has been reported for coal particles[6] but generally there is not much published work on this topic.

Routine shape factor measurements for particles smaller than about 36 British Standard mesh (500μ) will be undertaken reluctantly with such slow and tedious counting methods. The alternative automatic scanning procedures, though speeding the process, require expensive equipment. With this in mind a simple method for counting and weighing large numbers of closely sized particles was developed.

The original procedure[7,8] used a sieve, which was the limiting screen for the closely sized particles under investigation, carrying on its underside a weighed strip of 'Cellotape' of known area. Consequently, the number of covered apertures could be calculated. This will be referred to in the following as the 'working surface'.

The particles of known density were dusted over the working surface and generally one particle per aperture was retained by the adhesive film on the 'Cellotape'. However, a few exceptions were noted. If a wide range of shapes occurred within a size fraction it was found that the number of particles per aperture varied from none to two or three. While these frequently averaged one per aperture, as a refinement a few sample rows were counted by scanning with a travelling microscope. Since the particles were arranged in a rectangular matrix this proved a simple operation. With the larger particle sizes a simple precaution was necessary; because the 'Cellotape' must cover an integral number of apertures it was therefore preferable to measure the effective area of the working surface after dusting with particles. Carefully removing the 'Cellotape' with its adhering particles and re-weighing enabled the average weight and volume of a single particle to be calculated. Thus, k may then be determined directly, and f may be calculated from specific surface area data.

While the application of a mesh in this manner for particle counting was found to be a most useful innovation (it has since been discovered that the idea of counting by using a specially moulded plate holding one particle per recess is sometimes employed when bottling pharmaceutical products such as aspirin), the two main steps: (*a*) measuring the area of the 'Cellotape' for each determination; (*b*) peeling the 'Cellotape' away from the sieve for re-weighing, were inconvenient and the original method has been improved.

Disadvantage (*b*) is avoided by using rimmed British Standard mesh disks of about 7-cm. diameter. These weigh about 20 gm. and consequently the entire disk and 'Cellotape' with the adhering particles *in situ* can be weighed on an analytical balance.

Disadvantage (*a*) is not as easy to overcome as (*b*) but fortunately it is not as serious. However, if a mesh were available containing a known standard number of apertures then the entire surface could be covered with 'Cellotape' and area determination would be unnecessary. Utilizing this idea it was found that mesh rectangles were easily cut to give a working surface containing a standard number of apertures. However, these meshes possessed little mechanical strength, becoming both easily distorted and frayed at the edges. Sealing the edges to a depth of several aperture diameters with a plastic material, while still retaining a rectangular working surface, completely obviated these disadvantages.

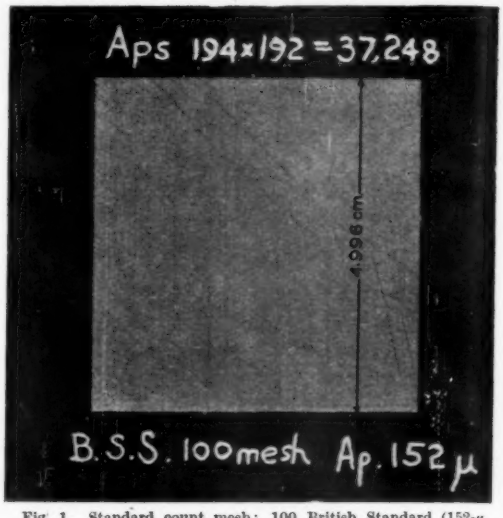

Fig. 1. Standard count mesh: 100 British Standard (152-μ aperture) containing 37,248 apertures

The technique of making these meshes is to use 'Cellotape' to avoid sealing the working surface when the plastic is applied to the edge and is given in the Appendix. Fig. 1 illustrates a standard aperture count mesh made from a 7·5-cm. square of 100 British Standard mesh (152-μ aperture, 102-μ wire diameter). Its edges were sealed to a depth of about 40 apertures (~ 1 cm.) and the working surface contains 37,248 apertures. Fig. 2 shows a magnified section near a corner of this mesh after dusting with coal particles sized between 100 and 120 British Standard mesh (152–124μ). This incidentally illustrates how sieves tend to select some coal particles along a diagonal and indicates that the ratio between the statistical diameter and the mean sieve aperture should be $\sim \sqrt{2}$. Values reported by Needham and Hill[6] support this.

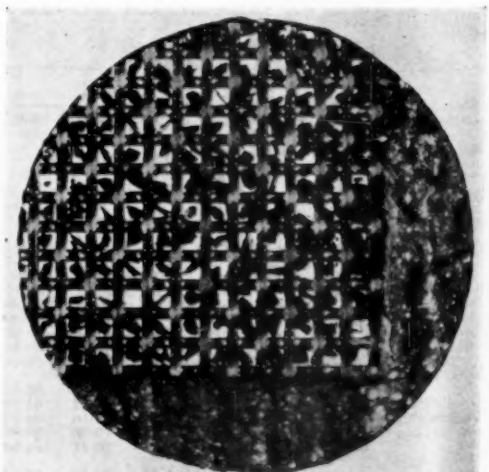

Fig. 2. Corner of mesh in Fig. 1 after dusting with 100–120 British Standard mesh coal particles (scale: aperture 152μ, wire diameter 102μ). Photograph taken by transmitted and reflected light. The highlights caused by reflexion of light from the wires have been reduced in intensity

This technique becomes difficult with meshes finer than about 100 British Standard. However, having sealed the edges, even though the working surface may not be rectangular, and consequently estimating the number of apertures becomes laborious; once the number is counted the operation need never be repeated. A photographic enlargement of the mesh is useful here.

For counting the finer particles, electrolytically deposited grids have been used. These have excellent mechanical strength and can be cut easily to produce a standard rectangular working surface. Sealing the edges is not necessary as it is with meshes and this is a decided advantage. However, preliminary results indicate that they are slightly inferior to woven mesh, for often two particles occupied one aperture. The grid is thinner than the correspondingly sized mesh, and its wires are rough and rectangular in section as against smooth and circular; observation indicates that these factors may have some influence on the adverse effects noted. Nevertheless, a technique for using these grids could probably be developed.

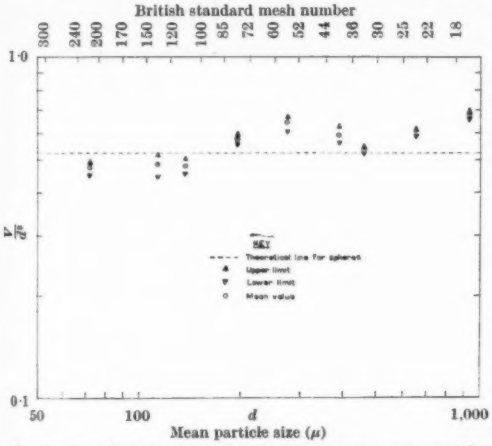

Fig. 3. Relationship between shape factor and mean particle size: coal sample

It is suggested that suitable fine meshes could be specially woven for this purpose. These would contain a standard number of apertures to British Standard or any other specification surrounded by a closely woven edging which would serve both to suitably terminate the working surface and to strengthen the mesh. A large number of these standard aperture count meshes could be woven simultaneously as one piece of cloth, the 'pattern' being (say) 5-cm. squares of working surface separated by 1-cm. wide stripes of close weave. These could be cut and suitably mounted. It should be noted that for 100 British Standard mesh material a 5-cm. square of working surface would contain about 40,000 apertures.

Preliminary results were reported earlier[7,8] where with a particular coal sample an average of $k = 0{\cdot}54$ was obtained. Interesting confirmation of this value based on Heywood's work has since been communicated (Bramley, J., private communication, reproduced in ref. 8).

Some recent measurements on coal samples are illustrated in Fig. 3, which demonstrates a variation in k with particle size. The method of plotting is clearly more revealing to changes in k than the usual logarithmic plot of V against d. Also, if a differently defined measure of particle size is introduced as a ratio with d then a plot by dimensionless groups follows; this could be useful in correlating shape factor data.

This work is part of a project concerned with the moisture retention properties of fine coal.

I wish to thank Prof. J. T. Whetton and my colleagues in this Department for interest, and especially Mr. D. Howitt, who carried out the experimental work.

COLIN C. HARRIS

Department of Mining,
University of Leeds. March 28.

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Appendix. A Method for making Standard Aperture Count Meshes

(1) Commence with a suitably sized mesh rectangle which has been cleaned in a grease solvent. A 7·5-cm. square was used here.
(2) Stick a strip of opaque 'Cellotape' on to the mesh so that the outer edge of the tape lies along and covers a wire situated about 1 cm. from the edge of the mesh. Repeat on the reverse side of the mesh for the same wire.
(3) Repeat the operation for the opposite edge of the mesh.
(4) Apply a layer of plastic material to both sides of one uncovered edge and quickly spread evenly on both sides with a spatula. Treat the opposite edge in the same way. An epoxy resin and a proprietary brand of plastic solder have both been used successfully.
(5) When the plastic is dry, but still soft, peel the 'Cellotape' away and a straight clean edge should result. To assist this it is sometimes advantageous to score the plastic gently with a sharp knife.
(6) When the plastic has hardened treat the remaining edges in the same way.

D. HOWITT

Gallium Arsenide as a Semi-insulator

SOME semiconductors have comparatively low resistivity, and this is due to their containing electrically active impurities with energy-levels near a band edge. Others have very high resistivity and this is due to their containing a predominance of impurities with levels far from the band edges, that is, far compared with the thermal energy kT. These latter materials are often referred to as insulators, but since they can carry electronic currents we prefer to introduce the term 'semi-insulator' to describe them. A material such as cadmium sulphide, which by suitable choice of impurity content can be made a semiconductor or a semi-insulator, is of particular interest and much work has been done on cadmium sulphide in the past few years. Gallium arsenide is another substance with the same property, but has the advantage that both n- and p-type material may be readily prepared.

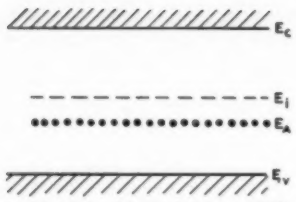

Fig. 1. Energy-levels of the model to be considered

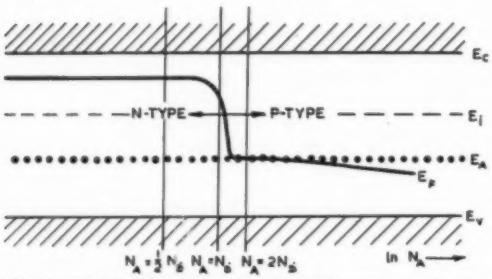

Fig. 2. Fermi-level as a function of acceptor concentration for fixed donor concentration (schematic)

As normally prepared in this laboratory undoped gallium arsenide is n-type with about 10^{16} electrons/cm.^{3}, which come from a residual impurity acting as a shallow donor. Under conditions in which oxygen is present during the preparation high-resistivity material is obtained. The most reasonable hypothesis is that oxygen gives rise to a deep acceptor-level which compensates the donors. It is therefore of interest to consider the behaviour of a system with a given concentration of donors N_D and a variable number of deep acceptors with concentration N_A. My experiments suggest that the acceptor-level at E_A lies below the intrinsic Fermi-level E_i, as shown in Fig. 1. The Fermi-level varies with N_A in the manner shown schematically in Fig. 2. It will be of advantage to introduce a quantity:

$$\chi \equiv \frac{N_A}{N_D}$$

which I shall call the 'degree of compensation'. Four regions of interest may be distinguished:

(1) $N_A \ll N_D$, $\chi \ll 1$. The concentration of electrons in the conduction band n is, to a good approximation, equal to N_D and the material is low resistivity n-type.

(2) $N_A \simeq N_D$, $\chi \simeq 1$. The Fermi-level drops from a position near the conduction band to one near the acceptor-levels, this change occurring over a very small change of N_A. For example, if N_A is 0·9 N_D then n is 0·1 N_D and the material is still of fairly low resistivity, the Fermi-level having dropped from its position in region 1 by only kT ln10 (about 0·06 eV. at room temperature), while if N_A is 1·1 N_D then the Fermi-level has dropped to a position only kT ln10 above the acceptor-level, and n will decrease by many orders of magnitude. In the present model which has E_A below E_i the material will change from high resistivity n-type to high resistivity p-type in this region. When N_A is greater than but not much greater than N_D the electron concentration is given by:

$$n \simeq \frac{1}{\chi - 1} \cdot N_c \cdot \exp \frac{E_A - E_c}{kT} \qquad (1)$$

that is, it is determined by the degree of compensation and the acceptor energy-level only. From the absolute magnitude of n and its variation with temperature both χ and $E_c - E_A$ may be determined.

(3) $N_A > N_D$, $\chi > 1$. As soon as N_A becomes appreciably greater than N_D the Fermi-level becomes locked near the acceptor-level and the material is high resistivity p-type. In this region the hole concentration is given by:

$$p \simeq (\chi - 1) \cdot N_v \cdot \exp \frac{E_v - E_A}{kT} \qquad (2)$$

It should be noted that the range of validity of equation 2 is the same as that of equation 1, for both hold when the position of the Fermi-level is determined by N_A and N_D alone, p and n both being negligible.

(4) $N_A \gg N_D$, $\chi \gg 1$. The position of the Fermi-level is now determined by the distribution of electrons between the valence band and the impurity-level. As N_A increases the hole concentration increases, but since E_A is far from E_v then p cannot be large and the material is still high resistivity p-type. In this region:

$$p \simeq (N_v N_A)^{\frac{1}{2}} \exp \frac{E_v - E_A}{2kT} \qquad (3)$$

By considering Fig. 2 it is easy to see what modifications to this behaviour are introduced if E_A lies above E_i. The general shape of the curve of E_F against N_A is unchanged but now the point of intersection of E_F with E_i is moved to the right, that is, the change from high resistivity n-type to high resistivity p-type occurs at a much higher acceptor concentration, and in practice this may not be attainable.

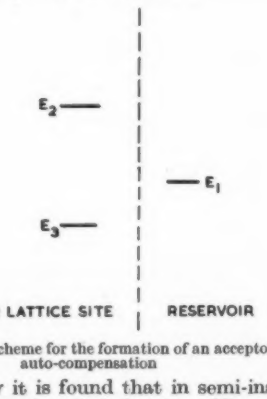

Fig. 3. The energy scheme for the formation of an acceptor during auto-compensation

Experimentally it is found that in semi-insulating gallium arsenide E_A does lie below E_i. The existence of n-type high resistivity material requires that $\chi \sim 1$ and we find from the experiments, using equation 1, that $\chi - 1$ is as little as 10^{-5}. Clearly it is unlikely to happen in a large number of specimens that N_A should approach N_D so closely just by chance, and some mechanism of automatic compensation must be operative. A rather general model for this is illustrated in Fig. 3. Again we regard N_D as a fixed given quantity. There is a reservoir of lattice defects (in which term I shall include impurities) which are in an electrically inactive state at energy E_1. These can go over to lattice sites at which they behave as acceptors, with energy E_2. If in this site they accept an electron the energy of the crystal decreases by an amount $E_c - E_A$, and this state has energy E_3. One then has a problem in statistical thermodynamics in which defects are distributed over levels E_1, E_2, E_3 and electrons over levels

E_v, E_A, E_c, the two systems being coupled by the fact that the level E_2 has an electron associated with it. To investigate this model in detail requires a more exact specification of the states; but it is immediately obvious that if the energy differences $E_2 - E_1$ and $E_1 - E_3$ are both large compared with kT and the entropy difference between the states is not too great then almost exact compensation will occur as long as the reservoir contains sufficient defects. In other words, it is energetically advantageous for defects to become acceptors so long as there is an electron for them to accept, but not otherwise.

An alternative possibility is that acceptors and donors form associated pairs[1], but this mechanism should lead to an increase in electron mobility, contrary to observation. Also the calculated degree of pairing is insufficient to account for the exactness of compensation found.

In the case of semi-insulating gallium arsenide it seems probable that oxygen is dissolved in the lattice in two different sites, being electrically active in one site (as an acceptor) and inactive in the other (as the reservoir). In the case of cadmium sulphide and similar compounds, the work of Kröger and Vink[2] shows clearly that the deep levels are associated with crystal defects arising from non-stoichiometry, and the reservoir of such defects is obviously the ambient atmosphere. In cadmium sulphide one can vary χ by annealing crystals at various temperatures and in various atmospheres, and similarly in gallium arsenide one should be able to vary χ by suitable heat treatment.

My model suggests that semi-insulators of the type I have described, that is, those in which a shallow donor is auto-compensated by a deep acceptor, should exhibit pronounced hole trapping effects but little electron trapping. This has a number of practical implications. For example, the high photoconductivity of these materials is readily explained. Also the existence of large space-change currents in certain crystals of cadmium sulphide[3] but not in others may be explained by variations in χ between the crystals. One might predict that semi-insulating gallium arsenide suitably heat-treated might also show such currents.

The ideas expressed here were developed while attempting to account for the experimental results of Dr. G. T. Wright on cadmium sulphide and of Dr. C. Hilsum and his colleagues on gallium arsenide. I wish to thank them both for extensive discussion of the problems involved.

Acknowledgment is made to the Admiralty for permission to publish this communication.

J. W. Allen
Services Electronics Research Laboratory,
Baldock, Herts.

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Gallium Arsenide for γ-Ray Spectroscopy

Crystal conduction counters offer an improvement in energy resolution over ionization chambers or scintillation counters for spectroscopy of nuclear particles. Semiconductor counters which employ a p–n junction are useful only for heavy charged particles of moderate energy. For energetic charged particles or γ-rays with ranges exceeding 1 mm. of silicon a crystal with efficient charge collection throughout its bulk is required. Most crystals of sufficiently high resistivity contain so many electron traps that collection of the ionization current is inefficient, and only diamond has previously been found to count single γ-ray events. The usefulness of diamond as a crystal counter is restricted.

We have found that the semiconductor gallium arsenide with resistivity at room temperature exceeding 10^6 ohm-cm. is an efficient particle counter. When a bar of this material is bombarded with γ-radiation from cobalt-60, pulses of charge are readily observed. The time for the pulse-rise is about 5 μsec., and pulses of 0·5 mV. across a megohm load are seen with an applied field of 200 V./cm. This effect occurs in both n-type and p-type gallium arsenide of high resistivity. The samples used in these experiments were of dimensions 1 cm. × 0·2 cm. × 0·1 cm., but there appears to be no reason why larger crystals should not be made. We are examining the spectrum of the pulse-height and resolution of energy, and our results will be reported elsewhere.

Acknowledgment is made to the Admiralty for permission to publish this communication.

W. R. Harding
C. Hilsum
M. E. Moncaster
D. C. Northrop
O. Simpson
Services Electronics Research Laboratory,
Baldock, Herts. May 25.

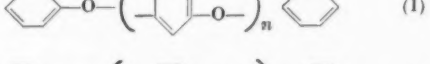
ENGINEERING

Pump Fluids for Higher Vacuums

In the decade after Burch introduced petroleum oils[1] as working fluids for the Langmuir condensation pump, the silicones, octyl alcohol esters and petroleum pump fluids converged to a low-pressure optimum at about 10^{-7} mm. mercury, which the succeeding 20 years has lowered, perhaps, to 5×10^{-8} in an untrapped three-stage glass pump when measured at an ambient temperature of 25° C.

Recently, particle accelerators and the conquest of space have made demands for pressures ten or a hundred times lower, to be induced in relatively enormous containers, and it has been necessary to augment the pumps with traps filled with liquid nitrogen or helium, together with means for periodic baking out. The proliferation of apparatus occasioned by these and other alternatives is becoming far too cumbersome and expensive.

In April 1959, learning of their use as high-temperature lubricants, I began examination of a class of phenoxy benzenes and polyphenyl ethers[2] of the general formulæ:


(1)

(2)

which may restore simplicity to the vapour pump. Not only can some of the compounds give pressures in the 10^{-8}–10^{-10} range untrapped, but also their tendency to wander through vacuum labyrinths is reduced many hundredfold over previously used fluids. Thus a Bayard–Alpert ionization gauge[3]

attached to a three-stage glass air-cooled pump (type 25*W*, Consolidated Vacuum Corp.) by a tube 2 cm. in diameter with two right-angle bends showed no clouding of the glass beyond the first bend in two weeks at an ambient temperature of 30° C. The filament, energized after 54 hr. inactivity, indicated an immediate pressure of 8×10^{-8}—the initial gas burst—and 1×10^{-9} 13 min. later, without resort to degassing or baking. The lowest indicated pressure with the filling :

m-bis(*m*-phenoxyphenoxy)-benzene (3)

was $4{\cdot}5 \times 10^{-10}$ mm. mercury, again untrapped. This is equivalent to an altitude above the Earth of 400–800 km. [4], according to latitude, and a mean free path of 10–100 km., depending on the composition of the residual gas.

Table 1

Name of pump fluid	Temperature in pump boiler (approximately)	Vapour pressure (25° C., mm. mercury) (extrapolated)	Lowest pressure (mm. mercury) yet recorded in untrapped pumps, 25° C., ambient	
			1-Stage*	3-Stage†
2-Ethyl hexyl sebacate	190° C.	2×10^{-8}	1×10^{-6}	8×10^{-8}
bis (*m*-Phenoxyphenyl) ether	195° C.	10^{-8}		
m-bis(*m*-Phenoxyphenoxy)-benzene	250° C.	10^{-12}	5×10^{-8}	5×10^{-10}
bis-*m*(*m*-Phenoxyphenoxy)-phenyl ether	280° C.	10^{-13}	5×10^{-8}	
Mixed meta- and para-5-ring ethers	250° C.	$< 10^{-12}$	5×10^{-7}	$1{\cdot}1 \times 10^{-9}$

* This experiment, tested in vertical glass pump, with *Vg.* 1*a* gauge tube.

† This experiment, tested in 3-stage glass fractionating pump, with Bayard-Alpert *G.I.C.* 110 tube.

The results of some early comparative tests are shown in Table 1, while the gas bursts at ultimate vacuum are recorded at an ambient temperature of 30° C. in Fig. 1. The single-stage pump was fitted with a *Vg.* 1*a* ionization gauge tube through two right-angle turns; and the three-stage pump with a Bayard–Alpert gauge *G.I.C.* 110. In each case, the gauge had been de-energized for 16 hr. after reaching a low-pressure plateau, and it is the response immediately after re-energizing the filament that is shown in Fig. 1. Other compounds, tested only in the single-stage glass pump, for lack of material, showed considerable promise, for example :

bis(*m*-(phenoxyphenoxy)-phenyl) ether (4)

which on one occasion reached an unreadable value with the *Vg.* 1*a* ionization tube. A 5-ring meta-para-isomer gave a very high vacuum in the three-stage pump, 1×10^{-9} or better. Since the vapour pressures at room temperature of the 5-ring ethers are of the order 10^{-12} mm., it is probable that further purification, coupled with modification of pumps, should lead to the direct production of still lower pressures. The ultimate vacuums of the present experiments were not immediately obtainable, but required treatment of the samples and generous insulation of the pumps to conserve heat and prevent charring of the bench with the higher boiling fillings. The behaviour in metal pumps and other vacuum properties of these fluids will be presented through the vacuum journals.

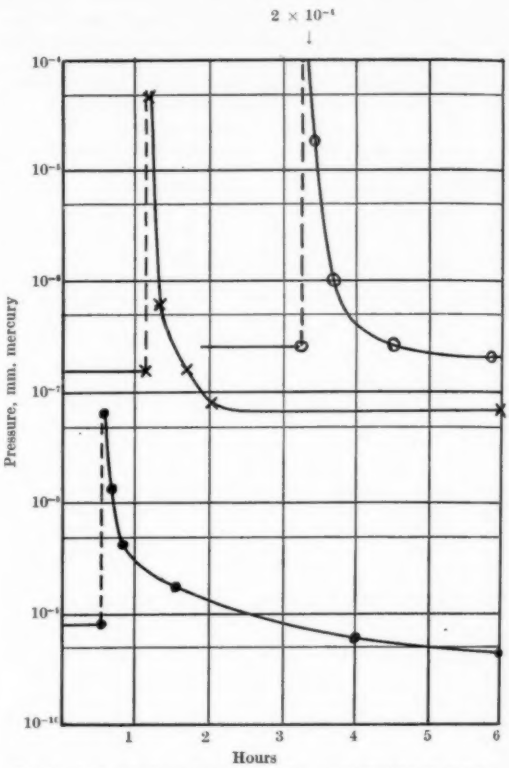


Fig. 1. ○, A petroleum base pump oil ; ×, 2-ethyl hexyl phthalate ; ●, *m-bis*(*m*-phenoxyphenoxy)-benzene

I am indebted to the Monsanto Chemical Co., St. Louis, Missouri, for gifts of the compounds, and to the Trans Penn Wax Corporation, Titusville, Pennsylvania, for contributory financial support. Correspondence concerning vacuum applications of the fluids may be addressed to me.

Kenneth Hickman

136 Pelham Road,
Rochester, 10,
New York.

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METALLURGY

The Sulphide Phase in Some Iron Meteorites

Metallic meteorites are composed in varying proportions of two iron–nickel metallic solid solutions, kamacite, which has a low nickel content, and taenite, which has a higher nickel content. The structure and distribution of these phases have been extensively examined on a macro- and on a micro-scale. Many iron meteorites also contain non-metallic inclusions of an iron–nickel phosphide (schreibersite) or an iron–nickel sulphide (troilite). Troilite has not been subject to very detailed microscopic examination for

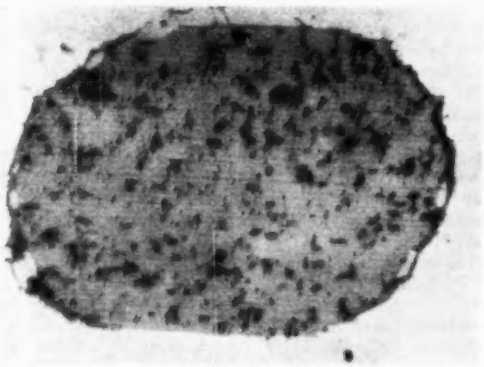

Fig. 1. Walker County unetched. (× c. 215)

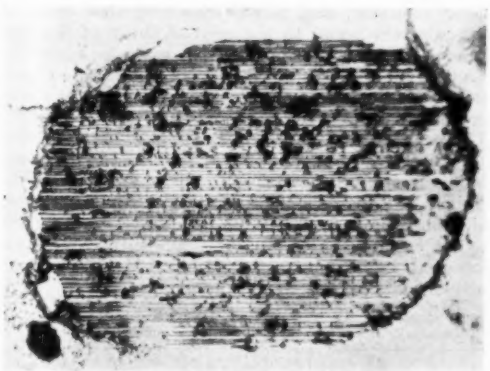

Fig. 2. Same area after sulphur printing. (× 215)

two reasons. First, it shows a great tendency to chip and disintegrate during preparation and, secondly, it is very reactive towards most etching agents; hence, when metallic meteorites are deeply etched for museum exhibition the troilite is usually protected from the etching agent by a suitable stopping-off compound.

Perry[1] has published a photograph of an anomalous lamellar structure which he found at a single location within a vein of terrestrial weathering material in the Walker County hexahedrite and which he identified as a kamacite–taenite aggregate. However, the general appearance and location seem to suggest that the structure might have arisen from a partly weathered non-metallic compound, hence a search for similar structures has been made in unweathered portions of Walker County and other hexahedrites. Several examples have been found in a small piece of Walker County loaned for study by the British Museum (*B.M.* 35413). Fig. 1 shows an area in the unetched condition. The groundmass is kamacite, in which is embedded a roughly circular brown patch of striated material. The black areas are holes due to the chipping out of material during preparation, and there are occasional patches of white schreibersite situated between the striated phase and the groundmass of kamacite. The striated phase proved to be sulphide bearing, as shown by its response to sulphur printing[2]. The same area examined immediately after sulphur printing shows the striations much more distinctly (Fig. 2) and reveals that the dark component of the striated pattern, which is undoubtedly troilite, is more heavily cracked than the light component. It is not possible at this stage to give a firm identification of the light striations.

Similar effects have also been obtained from small troilite areas in the hexahedrites Coahuila (*B.M.* 53295) and Tocopilla (*B.M.* 1931, 13). In each case the sulphide body was associated with a partial or complete rim of schreibersite.

H. J. Axon

Metallurgy Department,
University of Manchester.

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A New Phase in a Silicon bearing 17 per cent Chromium, 8 per cent Nickel and 1 per cent Niobium Steel

During the study of a 17 per cent chromium–8 per cent nickel–4 per cent silicon–1 per cent niobium steel, the composition of which is shown in Table 1, a phase not previously reported was extracted and subjected to X-ray examination.

An X-ray diffraction pattern was obtained from the extracted residue in a 19-cm. powder camera using cobalt radiation filtered with iron foil. The interplanar spacings of the phase contained in the residue are shown in Table 2. The pattern can be indexed on a tetragonal structure having parameters $a = 4{\cdot}806$ Å., $c = 5{\cdot}955$ Å. and a c/a ratio of $1{\cdot}238$. The presence of gelatinous silicic acid in the residue prevented an accurate chemical analysis being performed, although it appeared that a high concentration of niobium together with some chromium,

Table 1

Element	C	Si	Ni	Cr	Nb
Per cent	0·006	3·90	8·11	16·90	1·04

Table 2. *d* Spacings

Tetragonal a = 4·806 Å.; *c* = 5·955 Å.; *c*/*a* = 1·238

h,*k*,*l*	*d* calculated	*d* observed	Intensity
002	2·978	2·98	8
111	2·951	2·944	2
102	2·531	2·527	2
200	2·403	2·413	10
—	—	2·331	1
201	2·228	2·184	1
210	2·149	2·147	9
211	2·021	2·022	1
003	1·985	1·986	7
202	1·870	1·875	5
212	1·743	1·743	6
310	1·520	1·519	6
004	1·489	1·486	6
213	1·458	1·459	6
312	1·354	1·351	4
223	1·291	1·285	8
214	1·224	1·224	4
313	1·207	1·207	4
400	1·201		
005	1·191	1·191	1
401	1·178	1·180	2
420	1·074	1·074	5
314	1·063	1·063	6
215	1·042	1·043	4
403	1·028	1·021	7
422	1·011	1·011	1
333	0·984	0·982	6
500, 430	0·961	0·961	7
423	0·945	0·945	5
315	0·937	0·938	5
502	0·915	0·915	6

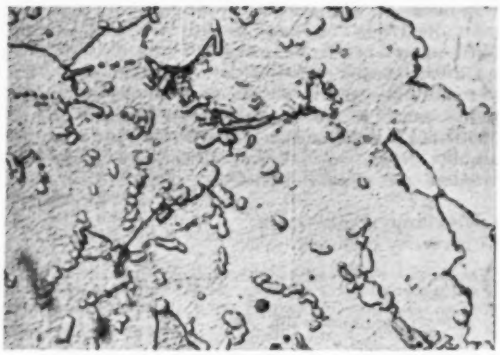

Fig. 1. Unidentified phase in 17/8/1 niobium + 4 per cent silicon alloy etched in Kalling's reagent. (×1,000)

silicon and carbon was present. The difficulty of extracting the phase under examination free from contamination by silicic acid prevented a density measurement being performed and so no space group could be assigned to the phase.

The appearance of the phase in the microstructure is shown in Fig. 1.

We wish to acknowledge that Dr. H. Hughes (United Steel Companies' Research Laboratory) pointed out the tetragonal nature of the X-ray pattern before it had been finally indexed.

M. J. O'Hara
D. Wilkinson
R. T. Allsop

G.K.N. Group Research Laboratory,
Birmingham New Road,
Lanesfield,
Wolverhampton.
May 30.

CHEMISTRY

Mass Spectrometric Investigation of the Vaporization of Sulphides and the Dissociation Energy of S_2

The disagreement between second- and third-law values of the dissociation energy of $S_2(D^\circ_0(S_2))$ obtained from equilibrium measurements was emphasized many years ago[1]. A relationship was also pointed out between the heat of combustion of SO, $D^\circ_0(O_2)$, $D^\circ_0(SO)$ and $D^\circ_0(S_2)$, which should permit one to choose one of the values allowed by observed predissociations for $D^\circ_0(S_2)$ and $D^\circ_0(SO)$. Recent measurements[2] do not seem to settle the question[3].

In the vapour phase above calcium sulphide we have observed by mass spectrometry the dissociation $S_2 \rightleftharpoons 2S$, and $CaS \rightleftharpoons Ca + S$, and have calculated $D^\circ_0(S_2) = 4{\cdot}4 \pm 0{\cdot}2$ eV., $D^\circ_0(SO) = 5{\cdot}4 + 0{\cdot}2$ eV., $D^\circ_0(CaS) = 3{\cdot}0 \pm 0{\cdot}2$ eV. and ΔH°_{298}(vap., CaS) = $6{\cdot}4 \pm 0{\cdot}2$ eV.

The mass spectrometric method has been described previously[4,5]. The sample was heated in a molybdenum Knudsen cell; provisions were made for distinguishing between residual gas molecules and those of the beam, which passes through a cooled collimation system; ion intensities were measured with an electron multiplier. Absolute values of partial vapour pressures were obtained from $P_i = I_i T/S_i$; I_i is the measured current corresponding to a species i, T the absolute temperature of the Knudsen cell and S_i the sensitivity for i (ref. 6): $S_i = (M_i/2\pi R)^{1/2}(s/G_i)\Sigma I_i T^{1/2}\,\Delta t$; M_i is its molecular mass, R the gas constant, s the area of the effusion orifice and t the time necessary to evaporate the weight G_i of species i. Brightness temperatures of a small threaded hole at the bottom of the Knudsen cell have been measured with an optical pyrometer; emissivity and window corrections were made[7].

Dissociation energies were calculated from: $D^\circ_0 = -RT\ln K + T\,\Delta((F^\circ_T - H^\circ_0)/T)$ (third law) and $D^\circ_T = -Rd\ln K/d(1/T)$ (second law) using free-energy functions taken from Stull and Sinke[8], or for calcium sulphide, calculated by comparison of oxides and sulphides, assuming $\omega_e = 390$ cm.$^{-1}$, $r_e = 2{\cdot}3$ Å. and that only the $^1\Sigma$ ground-state is important.

Ionization cross-sections were taken from Otvos and Stevenson[9], and it was assumed that those of molecules are equal to the sum of those of the constituent atoms. The mass effect and the molecular effect of the multiplier[10] were considered to compensate each other. A possible error of 20° in the temperature measurement would lead to an uncertainty of 12 per cent in the second-law and of 1 per cent in the third-law values; instead of the assumptions made above, it is reasonable to suggest that the product of ionization cross-section and multiplier gain is the same for the atom and for the molecule[11]: in that case the third law D°_0 values of Table 1 are increased by about + 3·5 kcal. and ΔH°_{298}(vap., CaS) diminished by the same amount.

Table 1

T ° K.	log p (S)*	log p (S_2)	log p (Ca)	log p (CaS)	D°_0 (S_2) (kcal.)	D°_0 (CaS) (kcal.)	ΔH°_{298} (vap., CaS) (kcal.)
1,922	−5·67	−6·27	−5·47	—	97·4	—	—
1,975	−5·17	−5·96	−5·11	—	93·9	—	—
2,026	−5·05	−5·72	−4·86	—	95·3	—	—
2,048	−4·75	−5·53	−4·67	—	93·7	—	—
2,053	−4·74	−5·54	−4·64	−6·86	93·7	68·2	152·6
2,070	−4·89	−5·59	−4·74	−6·63	96·0	73·2	149·7
2,098	−4·57	−5·34	−4·45	−6·59	94·5	68·8	152·2
2,143	−4·47	−5·19	−4·35	−6·26	96·0	71·6	152·3
2,144	−4·31	−5·09	−4·22	−6·32	93·8	68·2	152·9
2,154	−4·42	−5·15	−4·31	—	95·9	—	—
2,156	−4·35	−5·09	−4·18	−6·31	93·9	67·9	153·6
2,190	−4·23	−4·99	−4·07	−6·15	95·4	69·1	154·3
2,223	−3·98	−4·73	−3·90	−5·87	94·4	68·7	154·5
2,297	−3·69	−4·53	−3·56	−5·59	93·8	68·1	156·4
				Average	94·8	69·3	153·2†
				Slope method	108	92	129
				Proposed value	101 ± 5	70 ± 5	147 ± 10

* Decimal logarithms of pressures in atmospheres.

† $[F^\circ_T - H^\circ_{298}]/T$ for this process is based on very scanty data on C_p (CaS) (Kelley, K. K., U.S. Bureau of Mines Bulletin 476, Washington (1949)).

The results in Table 1 and ΔH°_{298}(vap., Ca) (ref. 8) = 42·2 kcal., $\Delta H^\circ_{298}(\frac{1}{2}S_2) = 15{\cdot}4$ kcal., the standard heat of formation ΔH°_{298f}.(CaS) = 110·0 ± 2 kcal. (ref. 12) permit one to calculate ΔH°_{298}(vap., CaS) = 147 kcal. The value obtained from this cycle is considered to be more reliable than that given in Table 1 (see note to Table 1).

$D^\circ_0(S_2)$ obtained here, combined with ΔH°_0(SO) (ref. 2*b*) for the reaction $\frac{1}{2}S_2 + \frac{1}{2}O_2 \rightleftharpoons SO$, definitely rules out a value $D^\circ_0(SO)$ smaller than 5·2 eV. There are good spectroscopic arguments for $D^\circ_0(SO) = 5{\cdot}358$ eV. (ref. 13) rather than 5·027 eV. (ref. 3). Our measurements are not accurate enough to permit

one to settle the question whether the predissociation at 4·41 eV. gives the exact dissociation of S_2 (ref. 14) or an upper limit[15]; a value $D(S_2) = 4·2$ eV. would agree with our results[16]. Within the accuracy quoted in Table 1, all available data on $D(S_2)$ and $D(SO)$ (ref. 3) except appearance potential measurements[17] and older equilibrium measurements (cf. refs. 1, 2, 3, 14) seem to be in agreement.

Experimental details and further data on strontium sulphide and barium sulphide will be published shortly.

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R. Colin*
P. Goldfinger
M. Jeunehomme

Laboratoire de Chimie Physique I,
Université Libre de Bruxelles,
Brussels.

* Boursier de l'I.R.S.I.A.

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Ionization and Attachment Coefficients in *n*-Alkyl Chloride Gases

We have measured ionization coefficients in the five *n*-alkyl chloride gases, methyl, ethyl, propyl, butyl, and amyl chloride, using an experimental apparatus similar to that described by Geballe and Harrison[1]. Despite the fact that these gases contain a halogen atom, they exhibit no detectable electron attachment to form negative ions. The present work constitutes part of an experimental programme for measuring ionization and attachment coefficients in a large variety of electronegative gases. We hope that such data will serve as a guide for the correlation of these coefficients, important to an understanding of the electrical breakdown of gases, with molecular structure.

The gases used in this work are commercially available products which, when necessary, were purified by vapour phase chromatography. We estimate the final purity of each gas to be greater than 99 per cent.

The essential part of the experimental apparatus consists of a pair of plane, parallel electrodes of 10 cm. diam., the spacing between which is variable from 0 to 3 cm. The cathode is irradiated with ultra-violet light through perforations in a 1-in. diam. area in the centre of the anode, to produce a constant primary electron photocurrent, i_0. The current to the anode, i, is measured as a function of the electrode spacing, δ, at constant field strength, E, and gas pressure, P. Pressures used varied from 1 to 40 mm. mercury.

In each of the alkyl chloride gases the current was found to vary as $i = i_0 \exp \alpha\delta$. From the absence of curvature in plots of log i versus δ over a range $0·1 < \delta < 3$ cm., we can estimate that if negative ion formation occurs at all, the coefficient of attachment, η, must be smaller than 10–15 per cent of the observed ionization coefficient, α, at each E/P in every gas. Furthermore, in methyl chloride, for example, at $E/P = 40$, $\alpha/P = 0·0307$ and $\eta/P < 0·005$. Since an electron capture process, for example, $e + CH_3Cl = CH_3 + Cl^-$, is usually characterized by an η/P which varies slowly with E/P [1], an upper limit to η/P for capture processes in methyl chloride can probably be set at approximately 0·005 for all the E/P range studied.

The absence of measurable attachment is consistent with the mass spectrographic studies of methyl chloride by Dibeler and Reese[2]. They found that negative ions are not produced by capture processes, but only by a double-ion process, $CH_3Cl + e = CH_3^+ + Cl^- + e$, which has a small probability (about 1 per cent for 75-V. electrons) relative to that for the production of CH_3Cl^+ ions.

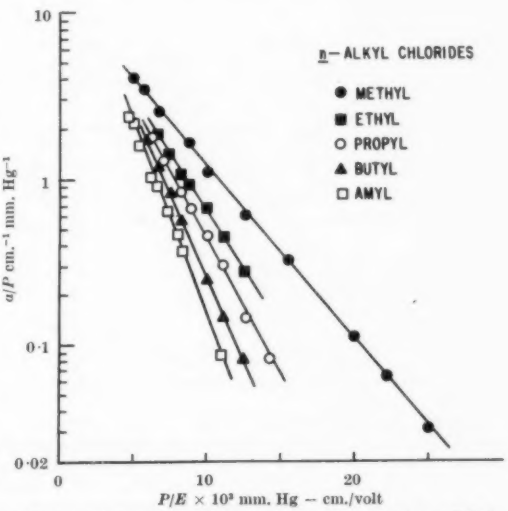

Fig. 1. Observed ionization coefficients as functions of P/E

The variation of α with E and P in the alkyl chlorides is expressed accurately by the functional form $\alpha/P = A\exp(-BP/E)$, as is illustrated in Fig. 1. It is especially to be noted that the functional form $\alpha/E = A\exp(-BP/E)$ will not fit the data. Values of the constants A and B, as derived from the curves in Fig. 1, are listed in Table 1.

Table 1

Gas	A cm.$^{-1}$ mm.$^{-1}$	B v cm.$^{-1}$ mm.$^{-1}$	ε_i eV.	$B/\varepsilon_i^{3/2}$ cm.$^{-1}$ mm.$^{-1}$ $v^{-1/2}$
CH_3Cl	13·4	244	11·28*	6·4
C_2H_5Cl	16·1	325	10·97*	8·9
n-C_3H_7Cl	20·7	388	10·8†	10·9
n-C_4H_9Cl	28·4	471	10·7‡	13·4
n-$C_5H_{11}Cl$	40·4	549	10·6‡	15·8

* Watanabe, K., *J. Chem. Phys.*, **26**, 542 (1957).
† Stevenson, D. P., and Hippel, J. A., *J. Amer. Chem. Soc.*, **64**, 2766 (1942).
‡ Estimated.

At present there exists no exact theory for the correlation of these quantities with molecular structure. The approximate theory of Crowe, Bragg and Devins[3] predicts that the constant B will vary with molecular parameters in a homologous series as $B = C\varepsilon_i^{3/2}\theta$, where ε_i is the molecular ionization energy and θ the total cross-section for electron scattering. Devins and Crowe[4] and Heylen and Lewis[5] derived values of B for a number of saturated hydrocarbon gases from sparking potential measurements. They found that $B/\varepsilon_i^{3/2}$ is directly proportional to the number of carbon–hydrogen bonds in the n-alkane gases, indicating that electrons in the carbon–hydrogen bonding orbitals (that is, the electrons in the outermost molecular orbitals) are most effective in electron scattering.

The values of $B/\varepsilon_i^{3/2}$ of Devins and Crowe for the alkanes are plotted in Fig. 2 along with our values for the alkyl chlorides. From the theory, the slope of the alkyl chloride curve is proportional to the C–H bond cross-section, while the intercept is proportional to the cross-section of chlorine bonded to a carbon atom. Analysis of the data yields a least-squares line of slope $1{\cdot}16 \pm 0{\cdot}03$ and intercept $2{\cdot}97 \pm 0{\cdot}07$. The slope for the alkyl chloride data is approximately twice that for the alkanes. The largest part of this difference is possibly due to an incorrect choice for the functional form for α by Devins and Crowe. The sparking potential data cover a rather limited range of E/P, so that their data can be fitted equally well using either $\alpha/P = A\exp(-BP/E)$ or $\alpha/E = A\exp(-BP/E)$. The B values they quote were obtained using the latter form. If the former expression were used instead, all of the B values for the alkanes would be larger by a multiplicative constant of approximately 1·5. Even so, the two slopes in Fig. 2 would still be different by 25 per cent, indicating either a larger C–H bond cross-section in the alkyl chlorides or different proportionality constants for the two homologous series in the equation $B = C\varepsilon_i^{3/2}\theta$.

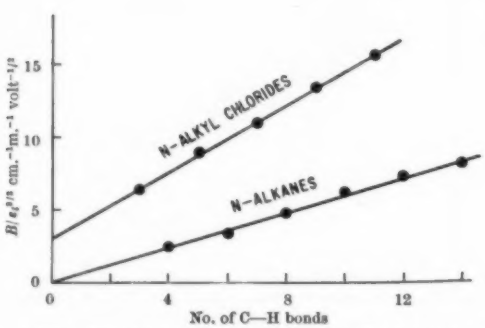

Fig. 2. Dependence of $B/\varepsilon_i^{3/2}$ on the number of carbon–hydrogen bonds per molecule. Upper curve, this work; lower curve, ref. 4

The constant A in the alkyl chlorides appears to vary as $a + b(n_{C-H})^3$. The significance of this observation is not clear.

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J. C. Devins
O. H. LeBlanc, jun.

General Electric Research Laboratory,
Schenectady,
New York.

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Homogeneous Synthesis of Isotactic Polystyrene using *n*-Butyllithium Initiator

Organolithium compounds are known to cause the stereospecific polymerization of dienes[1] and methacrylates[2]. Via n-butyllithium, styrene polymerizes easily to atactic polymer at ordinary temperatures[3,4]. We have now found that styrene may be polymerized at low temperatures, employing butyllithium as a soluble initiator, to crystallizable isotactic polystyrene.

Polystyrene prepared in a hydrocarbon medium at temperatures in the range of − 30° C. and below exhibits infra-red, X-ray diffraction and nuclear spin resonance spectra identical to those shown by isotactic crystalline polystyrene prepared via triethyl-aluminium–titanium trichloride catalysis. In the presence of ethers, however, only atactic polymer forms, even at − 78° C. Isotactic polymerization is much slower at − 40° (~ 5 per cent conversion in 48 hr.) in a 7 : 1 volume ratio styrene–toluene mixture than at − 78° in tetrahydrofuran (> 90 per cent conv. in 1 hr.). Butyllithium concentrations of 5–10 m.mol./l. were used.

A block copolymer of atactic and isotactic types of polystyrene may be prepared by adding an ether to a styrene polymerization proceeding at − 40° in toluene. The same result may be achieved by raising the temperature to 0° after polymerizing for a period of time at about − 40°. Blocks of a second monomer species (such as isoprene, alkyl methacrylates and acrylonitrile) on isotactic polystyrene can be prepared even though styrene conversion is far from complete[5].

R. J. Kern

Monsanto Chemical Co.,
Dayton 7, Ohio.
June 20.

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BIOCHEMISTRY

Presence of Neutral Carbohydrates in Human Aortic Tissue

In 1938, Hisamura[1] demonstrated that demineralized bone tissue contained two carbohydrate-protein fractions, a uronic acid-containing and a uronic acid-free fraction.

Consden[2] and Glegg *et al.*[3] later showed that connective tissue from different organs contains both acid mucopolysaccharides and considerable amounts of neutral heteropolysaccharides. The designation neutral heteropolysaccharides covers neutral mucopolysaccharides, containing hexosamine, and neutral monosaccharides, as well as glycoproteins which have bound hexosamine and/or various neutral monosaccharides in a firmly covalent bond to the protein. The term neutral refers only to the absence of hexuronic acids as constituents of the molecule.

Bertelsen and Jensen[4] showed in an earlier publication that the content of acid mucopolysaccharides in aortic tissue increases with age. They also found with increasing age a deposit of ground substance which—after oxidation with periodic acid—was strongly stained with Schiff's reagent.

This communication reports preliminary results obtained from experiments made in an attempt to isolate neutral mucopolysaccharides from macroscopically normal aortic tissue.

An extraction was made with 0·5 *N* sodium hydroxide for 4 days in an ice-box. The aortic tissue had already been rid of adventitia, disintegrated and defatted. After neutralization of the filtrate a precipitate was deposited in two volumes of absolute alcohol (fraction I). According to Meyer and Chaffee[5] this fraction contains the acid mucopolysaccharides. By increasing the alcoholic percentage to 84 a further precipitate (fraction II), which according to Glegg *et al.*[3] contains uronic acid-free carbohydrates, was deposited.

Table 1 shows that the ratio fraction I/II increases considerably with age. Both fractions increase, but the percentage increase of fraction I is much larger than that of fraction II.

Table 1. Fractions expressed in Percentages of Dry, Defatted Weight

Age	Fraction I	Fraction II
7 months	3·3	3·1
2 years	5·3	4·2
45 years	11·4	4·9
58 years	14·1	5·4
60 years	14·6	6·2

The qualitative determination of uronic acid was made by carboxylic reaction. The monosaccharides were determined by their colour reactions to cysteine, carbazol and sulphuric acid, as described by Dische[6] (Table 2). The content of hexosamine in the fractions was determined quantitatively by Blix's[7] modification of Elson and Morgan's method after hydrolysis.

Table 2

Age	Uronic acid	Hexosamine	Ketohexose	Aldohexose	PAS
Fraction I					
7 months	+	+	÷	÷	(+)
2 years	+	+	÷	÷	(+)
45 years	+	+	÷	÷	(+)
58 years	+	+	÷	÷	(+)
60 years	+	+	÷	÷	(+)
Fraction II					
7 months	÷	+	+	+	+
2 years	÷	+	+	+	+
45 years	÷	+	+	+	+
58 years	÷	+	+	+	+
60 years	÷	+	+	+	+

The quantitative content of hexosamine determined in fractions I and II respectively, and converted into 100-gm. dried, defatted tissue, is given in Table 3. An unmistakable increase of the hexosamine contents with age will be observed in both fractions. The increase in percentage is by far the greatest in the fraction containing the acid mucopolysaccharides.

Table 3. Hexosamine Content (mgm. per 100-gm. Dried, Defatted Tissue)

Age	Acid mucopolysaccharides	Neutral mucopolysaccharides
7 months	262	128
2 years	358	137
45 years	550	156
60 years	625	176

The periodic acid–Schiff reaction was carried out according to the method indicated by McManus and Hoch-Ligeti[8]. Fraction II showed a very strong reaction, whereas fraction I only showed a faint reaction. There was a distinct difference between the ways in which the fractions were soluble in water. Fraction I proved slowly soluble, forming fairly viscous solutions; whereas fraction II was quickly soluble, with a viscosity similar to that of water.

The preliminary result shows that with increasing age a considerable deposit of mucoid substance occurs in aorta; acid mucopolysaccharides, forming the mucoproteins, and neutral mucopolysaccharides, forming glycoproteins similar in nature to serum proteins.

The increase of acid mucopolysaccharides is greater than the increase of glycoproteins, which is in complete agreement with the histological picture.

Histochemical experiments made on aortic tissue, concurrently with the biochemical experiments, were found to be in agreement with the above-mentioned results. A big increase in the content of acid mucopolysaccharides, starting even in childhood, was found. The deposit of periodic acid–Schiff-positive substance was not distinct in the adult aorta until after the second decade, and then it increased gradually with the years.

Sv. Bertelsen

Department of Pharmacology,
20 Juliane Maries Vej,
University of Copenhagen.
March 21.

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Organic Matrix of Tooth Enamel

Little is known about the chemical nature of the organic matrix of enamel, although it presumably plays an essential part in the initiation and organization of the growth of hydroxyapatite crystals. The mature enamel finally resulting from these processes is the hardest and most highly mineralized of verte-

brate tissues. Other mineralized tissues, bone[1] and dentine[2], contain 15–26 per cent of persistent organic matrix, at least 90 per cent of which is collagen. Mature human enamel contains only some 0·6 per cent of organic matter and 0·36 per cent of proteins, approximately half of which are soluble in acid decalcifying fluids[3]. Investigations of the composition of enamel proteins have been largely confined to insoluble residues from the demineralization of the mature tissue[3–5]. A recent finding, in this laboratory, that partly mineralized enamel from developing teeth in the human fœtus contains approximately 20 per cent of material of protein character was followed by a preliminary investigation of its amino-acid composition.

The upper central incisors were carefully removed from a 33-week old fœtus and the calcified caps, which consist of both enamel and dentine, were freed from soft tissues. The tooth caps were gently wiped with cotton wool, slightly moistened with saline solution, to remove traces of blood. The enamel was readily removed from the underlying dentine by gently chipping with a dental excavator, the procedure being carried out under an ophthalmic operation microscope. The enamel was thereby reduced to a white crystalline powder, which had a somewhat waxy texture and showed evidence of prism structure with the optical microscope. The enamel was dissolved in 6 *N* hydrochloric acid and the solution heated at 100° C. for 48 hr. to hydrolyse any proteins. After removal of the hydrochloric acid, the amino-acids were determined by a semi-micro, resin chromatographic method[6], loads equivalent to 0·8 mgm. of protein being transferred to 0·4-cm. diameter 'Dowex 50' columns.

The results, expressed as numbers of amino-acid residues per thousand total residues, are summarized in Table 1. No peaks were observed corresponding to hydroxyproline and hydroxylysine, which are characteristic constituents of collagen. It was concluded that no collagen was present and that the enamel had been adequately separated from dentine. The composition differed markedly from collagen[7], epidermal 'keratin'[8], elastin[9], blood proteins[10] and the insoluble proteins of mature enamel[3–5]. The overall composition was very characteristic and no similar results for another protein have so far been found in the literature. Characteristic features are: (1) proline accounts for one-quarter of the total residues; (2) the high ratio of glutamic to aspartic acid; (3) histidine is the predominant basic amino-acid; (4) tyrosine greatly exceeds phenylalanine; and (5) the high methionine content.

Table 1. Amino-acid Composition of Developing Enamel and Insoluble Protein from Mature Enamel
(Values are given as numbers of amino-acid residues per thousand total residues)

	Developing enamel from human fœtus	Acid-insoluble residue from mature human enamel
	Present work	Hess *et al.* (ref. 4)
Alanine	20·3	111
Glycine	65·0	304
Valine	39·6	33·8
Leucine	91·3	41·9
*iso*Leucine	32·7	16·3
Proline	251	47·0
Phenylalanine	23·4	45·8
Tyrosine	53·4	6·9
Serine	62·5	75·5
Threonine	38·1	44·8
Half-cystine	<4	6·1
Methionine	42·3	8·3
Arginine	23·3	47·0
Histidine	64·5	9·0
Lysine	17·7	31·2
Aspartic acid	30·3	45·8
Glutamic acid	142	78·0
Hydroxyproline	0	47·3
Hydroxylysine	0	—
Amide	146	—

The most complete data for the insoluble protein from mature enamel due to Hess *et al.*[4] (Table 1) are in reasonable agreement with the results of Battistone and Burnett[5] but show differences from those of Stack[3]. All three analyses, however, revealed significant amounts of hydroxyproline which, together with the glycine and alanine values, suggest that collagen is present. The cystine, serine and basic amino-acid values are compatible with the presence of an epithelial keratin, similar to that found in epidermis[8], in addition to collagen. The increased quantity of cystine found in the cuticle of enamel[11] may indicate that the protein of epidermal type is associated with the epithelial layer.

The existence of a high proportion of amino-compounds in hydrolysates of partially mineralized enamel suggests the original presence of proteins rather than free amino-acids or small peptides. The amino-acid composition of developing enamel differs remarkably from that of the insoluble protein fraction from mature enamel (Table 1). It is considered that this difference results from the developing enamel containing one or more proteins associated with the true enamel matrix, in which hydroxyapatite crystals are formed. The large differences in composition suggest that the matrix protein accounts for a substantial proportion of the total protein of developing enamel. It is not surprising that the matrix protein should be unlike the keratins in composition since it is secreted by highly active ameloblasts, whereas the epithelial keratins result from cell degeneration. The decrease in protein content from 20 per cent in fœtal enamel to 0·36 per cent in the mature tissue, together with the change in composition, suggest that the matrix protein is removed either during maturation of enamel, after eruption of the teeth or during subsequent aqueous extraction of the ground enamel. Removal of organic matrix in the later stages of enamel development would seem to be consistent with, and may be essential for, the attainment of a high degree of mineralization. This view is supported experimentally by the widespread finding that in sections of teeth decalcified with strong acids, structure attributable to the enamel matrix is visible in the earlier stages of tooth development, whereas mature enamel is completely dissolved. A transition from one stage to the other has been observed in continuously growing teeth[12].

J. E. Eastoe *

Department of Dental Science,
Royal College of Surgeons of England,
London, W.C.2. April 5.

* Senior Leverhulme Fellow in Biochemistry.

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Starch-like Polysaccharide of *Streptococcus pyogenes*

MANY strains of Lancefield group *A* streptococci synthesize a starch-like polysaccharide when grown on a medium containing maltose or maltose-polymers[1], and some of these strains also produce an extracellular amylase[2]. It has been shown that amylase-negative starch-accumulating strains are associated with acute glomerulonephritis and acute rheumatic fever[3]. There is as yet no direct proof that the starch itself is more than a metabolic marker of bacterial variants, but because of the possibility of its production *in vivo* its isolation and characterization were of considerable interest, for it has been shown that bacterial polysaccharides of high molecular weight may have long persistence in the tissues[4].

The group *A* strain, *IRP* 118, type 19, was grown in 15 litres brain–heart–infusion broth, with repeated additions of maltose and automatic adjustment to *p*H 7·4 during 48 hr. Thrice-washed cell suspensions (about 40 gm. dry weight) were extracted for 1 hr. at 0° with 400 ml. perchloric acid. The final perchloric acid concentration used differed in different experiments, but although the yield of polysaccharide was greater with 5 *N* acid than with 2 *N* acid, the properties of the products were very similar. After clarifying by centrifugation, the extract was carefully neutralized at 0° with sodium hydroxide to *p*H 6·0. On saturation of the neutralized extract with *n*-butanol and allowing to stand for two days at 0°, a small amount of a white precipitate appeared at the interface, but this did not stain with iodine and was therefore discarded.

The polysaccharide was then precipitated as a complex with iodine, iodine (0·1 *N*) in potassium iodide (0·3 *N*) being added to the aqueous layer until no more blue precipitate appeared and the supernatant solution was just yellow with excess iodine. (Further addition of iodine produced a brown turbidity.) The purple precipitate was washed with dilute iodine solution and then suspended in warm distilled water and just decolorized with dilute sodium thiosulphate. The polysaccharide was then precipitated from this solution by successive additions of acetone at 0°, the bulk of the iodine-staining material being precipitated between 35 per cent and 45 per cent v/v acetone. The product was washed with *n*-butanol and then with acetone and ether. The amorphous white powder thus obtained was poorly soluble in water, and solutions were best obtained by dissolving in 0·3 *N* sodium hydroxide and only neutralizing immediately before use.

It was shown by paper chromatography that the sole sugar produced by acid hydrolysis[5] of the product was glucose. Reducing-sugar estimation[6] after acid hydrolysis, however, showed that the product was only 95 per cent polysaccharide, the remainder being apparently inorganic salts. When calculations were based on the polysaccharide content it was found that hydrolysis with salivary α-amylase resulted in a 100 per cent yield of maltose (as estimated by the method of Somogyi[6]) and 100 per cent hydrolysis based on iodine stain. With a commercial sample of purified β-amylase, 98 per cent maltose was liberated and the optical density of the iodine stain[7] fell to 3 per cent of its original value. Since the residual iodine stain was always of the same quality as that of the original material, it seems likely that this discrepancy was due to partial retrogradation. The 'blue value'[8] of the product varied from preparation to preparation as did the yield. For example, one preparation of 60 mgm. polysaccharide from 38 gm. dry weight cells had a 'blue value' of 0·65, and one of 200 mgm. from 49 gm. cells had a 'blue value' of 0·8. Controlled retrogradation of the latter product gave fractions with 'blue values' of 0·87 and 0·75 respectively and a trace of a red-staining fraction.

None of these fractions, however, showed any significant limit to β-amylolysis. It therefore seems that the polysaccharide extractable from this culture by perchloric acid is of the purely straight-chain amylose type with a range of chain-lengths from about 40 to 60 glucose units. (Based on 'blue values' for linear dextrins (Whelan, W. J., private communication).) This is confirmed by the fact that the product is completely hydrolysed when incubated in the presence of excess glucose with a preparation of amylomaltase obtained from the same strain of group *A* streptococcus according to the method of Monod and Torriani[9] for the amylomaltase of *E. coli mutabile* (*ML*).

Although the polysaccharide isolated does not have a very high molecular weight it exhibits a great tendency to retrograde and this retrograded form is very insoluble and might be expected to persist for long periods if it was in fact ever formed in tissues.

H. BAUM

Department of Biochemistry and Chemistry,
Royal Free Hospital School of Medicine,
8 Hunter Street,
London, W.C.1.

N. CROWLEY

Department of Bacteriology,
Royal Free Hospital,
Gray's Inn Road,
London, W.C.1.

[1] Crowley, N., and Jevons, M. P., *J. Gen. Microbiol.*, **13**, 226 (1955).
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Ubiquinone (50) and Ubichromenol in *Aspergillus fumigatus*, Fresenius

FOLLOWING the recent discovery[1–5] of a series of ubiquinones in various biological tissues, a useful system for studying their biosynthesis was sought. A strain of *Aspergillus fumigatus*, Fresenius (we wish to thank Prof. J. H. Birkinshaw for the gift of a specimen culture), studied by Anslow and Raistrick[6], was observed to secrete 3-hydroxy, 4-methoxy, 2,5-toluquinone (fumigatin) into the medium. This compound is closely related to the ring nucleus of ubiquinone and it seemed possible that the above organism might also produce sufficient ubiquinone for the study of its biosynthesis.

Cultures of the fungus were grown at 30° in Roux bottles, each containing 200 ml. Raulin–Thom medium at *p*H 3·8. After various intervals from 3 days to 8 weeks, the thalli from groups of 2 bottles were removed and examined for ubiquinone. In the preliminary experiments, the tissue was saponified directly in methanolic potassium hydroxide in the

Table 1. The Stimulatory Effect of Leucine on the Synthesis of Ubiquinone (50) and Ubichromenol in *Aspergillus fumigatus* Fres

Group	Medium				Thallus				
	Leucine concentration (per cent)	Final *p*H	Ether extract*		Dry weight (gm.)	Lipids (mgm.)	Ubiquinone		Ubichromenol (mgm.)
			Total (mgm.)	Fumigatin			(mgm.)	(μmol./gm. dry weight)	
A 1	0	3·0	245	+++	2·35	219	1·17	0·6	0·02
2	0·10	3·5	127	++	1·97	225	1·74	1·0	<0·02
3	0·25	6·0	39	0	1·83	225	2·07	1·3	0·17
4	0·50	7·3	54	0	1·35	171	2·00	1·7	0·33
B 1	0	2·0	—	+++	1·98	224	1·18	0·7	—
2	1·00	4·0	—	0	0·62	104	1·22	2·3	0·20

There were three Roux bottles in each experimental group but the yields after 7 days growth are expressed per bottle containing 200 ml. Raulin–Thom medium with initial *p*H = 3·8.

* The ether extract consists mainly of substituted quinones of which a variable fraction is fumigatin. There were approximately 30 mgm. fumigatin in each bottle of control medium.

presence of pyrogallol, and the unsaponifiable matter extracted with ether. In the later work, the lipids were extracted directly from the thalli using acetone, alcohol and ether in turn. The unsaponifiable material or the lipid extract was then chromatographed on a column of partially deactivated, acid-washed alumina (Brockmann Grade III) by a standard procedure[7] for ubiquinone.

A pale yellow zone with the same chromatographic behaviour and absorption band as ubiquinone ($\lambda_{max.}$272 mμ in *cyclo*hexane) was eluted from the column. A second chromatography on a column of silicic acid/'Celite' (2 : 1 w/w) enabled ubiquinone to be obtained from pooled samples in crystalline form. It had the following properties which are characteristic of ubiquinone (50)[3]: m.p. 48·5° C. (uncorr.), E(1%, 1 cm.) 272 mμ = 168, infra-red spectrum identical with that of ubiquinone (50). Chemical analysis gave percentages carbon, hydrogen and oxygen of 81·62, 10·90 and 7·47 respectively compared with theoretical values of 82·04, 10·42 and 7·54. The methoxyl contents of two different samples were 7·38 and 7·21 per cent respectively, which also agree with the theoretical 7·19 per cent for ubiquinone (50) within experimental error. The dry weight of the thallus and ubiquinone content (0·6 μmol./gm. dry wt.) were maximal after 7–10 days growth and declined thereafter.

This level of ubiquinone is comparable with that in animal tissues and some yeasts[5]. Ubiquinones from the latter, however, generally possess shorter isoprenoid side-chains with 30–45 carbons.

From the aqueous medium, fumigatin[6] and another quinone, believed to be spinulosin (3,6-dihydroxy-4-methoxy 2,5-toluquinone), have been isolated. The formation of these, however, can be reduced or suppressed by adding *l*-leucine to the culture medium. At the same time, the organism is stimulated to make a larger amount of ubiquinone and also its cyclic isomer, ubichromenol[7,8] (Table 1). Ubichromenol was first isolated from animal tissues, but it can also be produced artificially by the adsorption of ubiquinone on very strong alumina[8]. The material found in *Aspergillus fumigatus* is not an artefact, because it was obtained from the tissue by direct extraction with organic solvents and resolved from ubiquinone by a chromatographic procedure known to be incapable of converting ubiquinone into ubichromenol.

The amounts of ubichromenol available from the fungus were insufficient to permit full chemical analysis, but its ultra-violet absorption spectrum in ethanol and the isosbestic points for the bathochromic shift with alkali are identical with those of the pure material isolated from human kidney tissue. Furthermore, both show the same R_F value on reversed-phase chromatography. It is therefore reasonable to assume that the ubichromenol isolated is isomeric with ubiquinone (50).

The table of results shows clearly that the reduction in output of free quinone from the thallus in the leucine-supplemented medium more than accounts for the absolute increase in ubiquinone (50) and ubichromenol. So the leucine is altering the pattern of metabolism of the mould in a more fundamental way than merely providing a readily available pool of isoprenoid units for the synthesis of the side-chain molecules which become coupled to the nucleus.

Fumigatin and ubichromenol may be related to intermediates in the biosynthesis of ubiquinone. Full details of this work and experiments with ^{14}C-labelled substrates will be published elsewhere.

One of us (N. M. P.) is in receipt of a Department of Scientific and Industrial Research studentship.

N. M. Packter
J. Glover

Biochemistry Department,
University of Liverpool.

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Bradykinin-releasing Factor from *Bothrops jararaca* venom

It was first shown by Rocha e Silva, Beraldo and Rosenfeld[1] that the venom of the snake *Bothrops jararaca* contains a bradykinin-releasing substance. Holtz and Raudonat[2] verified that the bradykinin-releasing substance is precipitated with ammonium sulphate at the level of 60 per cent, is independent of the proteolytic activity on hæmoglobin and accompanies the coagulating activity. Hamberg and Rocha e Silva[3] observed that the bradykinin-releasing factor is heat-resistant and that the decrease of its activity in presence of prolonged heating is parallel to that of the coagulating activity and to the fall of esterase activity on L-benzoylarginine methyl ester.

Table 1

Fraction	Coagulating specific activity†	L-Benzoyl-argine amidase specific activity†	μgm. bradykinin* released per 100 μgm. protein
1	143	1,196	40
2	609	65	70

* Standard preparation of bradykinin (3 units/mgm.).
† As described by Henriques, Fichman and Henriques (ref. 6).

Recently, Contzen, Holtz and Raudonat[4] found that the bradykinin-releasing substance is dialysable and ultra-filtrable, being independent of the coagulating factor but being accompanied by the hydrolysing activity on L-benzoyl-arginine methyl ester.

In the experiments reported here we have attempted to discover if the bradykinin-releasing activity is connected to the coagulating or to the L-benzoyl-arginine amidase activity of the venom and also to purify the bradykinin-releasing substance. In a first experiment a purified enzyme obtained from the venom of *Bothrops jararaca*, the *Bothrops* protease *A* (ref. 5), which is highly active on L-benzoyl-arginine amide, and a partially purified preparation of the coagulating factor, obtained by precipitation with ammonium sulphate[6], were studied. Table 1 shows that the bradykinin-releasing factor is independent of *Bothrops* protease *A*, its activity being higher in the fraction that has the strongest coagulating activity. In fact fraction 1, which is about 18 times as active as fraction 2 in its hydrolysing activity on L-benzoyl-arginine amide, liberated less bradykinin than fraction 2. On the other hand fraction 2, which is about four times as active as fraction 1 in its coagulating activity, is about twice as active as fraction 1 in its bradykinin-liberating activity. In another experiment a more active preparation of coagulating factor obtained by ammonium sulphate precipitation followed by electrophoresis on a starch column[6] was found to be less active as a releaser of bradykinin than the crude venom used for the same preparation. It can be seen (Table 2) that the coagulating specific activities found were 357 and 1,000 respectively for the crude venom and the purified preparation, while the crude venom liberated 100 μgm. of bradykinin and the preparation 50 μgm. of bradykinin per 100 μgm. of protein. It was therefore concluded that the bradykinin-releasing activity is independent of the *Bothrops* protease *A* and coagulating activities of the venom. This does not exclude, however, the possibility that the bradykinin-releasing substance has some amidase or esterase activity, but if so it must be certainly weaker than that of *Bothrops* protease *A*.

Table 2

Fraction	Coagulating specific activity	μgm. bradykinin released per 100 μgm. protein
Crude venom	357	100
Coagulating	1,000	50

Table 3

Fraction	Percentage of saturation with ammonium sulphate	Amidase specific activity	μgm. bradykinin released per 100 μgm. protein
1	Crude venom	27	30
2	0·45	5	—
3	0·55	9	—
4	0·60	24	—
5	0·70	72	90
6	0·80	298	240
7	1·0	35	15

Table 3 shows the experiment in which attempts were made to purify the bradykinin-releasing factor. The preparation was made by heating the venom and fractionally precipitating the supernatant with ammonium sulphate as previously described for *Bothrops* protease *A* (ref. 5). All the fractions were dialysed against saline for 48 hr. and then tested for its bradykinin-releasing activity. The fraction precipitated at the level of 0·70–0·80 saturation with ammonium sulphate contained the peak of the activity, being eight times as active as the crude venom used for the preparation, that is, 100 μgm. of protein of crude venom liberated 30 μgm. of bradykinin while the same amount of protein from the fraction liberated 240 μgm. of bradykinin. The peak of activity of the bradykinin-liberating substance coincides with the peak of activity of *Bothrops* protease *A*, but as was shown above, the two activities are independent. Also in our conditions the bradykinin-releasing factor was not dialysable as observed by Contzen, Holtz and Raudonat[4].

Olga B. Henriques
Mina Fichman

Laboratory of Biochemistry,
Instituto Butantan,
C.P. 65, São Paulo.

W. T. Beraldo*

Department of Physiology,
Faculdade de Medicina da U.S.P.,
São Paulo. Feb. 5.

* Present address: Faculdade de Medicina da U.M.G., Belo Horizonte, Minas Gerais.

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Anti-Pneumococcus Type XIV Activity of Precipitins from *Ricinus communis* Seeds

A chemical structure closely similar to type XIV pneumococcus polysaccharide is probably the basic substrate used in the formation of the human A, B, H and Le[a] blood group specific substances[1]. Studies of the precipitation of the various blood group specific substances and related materials by *Ricinus communis* seed extract suggest that the activity of the *Ricinus* precipitin is directed towards the basic chemical framework of the human blood group substances[2–4]. It is thus possible that the *Ricinus* precipitin is actually specific for type XIV pneumococcus polysaccharide. The following experiments were carried out to investigate this possibility.

Purified pneumococcal polysaccharides of type II, VIII, IX, XII, XIV and XVII were made up as 1/1,000 (w/v) aqueous solutions and tested by the conventional precipitation technique against *Ricinus communis* seed extract prepared by the method of Boyd and Reguera[5], clarified by filtration through Whatman No. 42 filter paper and sterilized by Seitz filtration. Only type XIV polysaccharide was precipitated (Table 1).

Table 1. Precipitation of Pneumococcal Polysaccharides by *Ricinus communis* Extract

Polysaccharide	Dilution			Controls
	1/1,000	1/10,000	1/100,000	
Type II	0	0	0	0
VIII	0	0	0	
IX	0	0	0	
XII	0	0	0	
XIV	3	1	tr	
XVII	0	0	0	

3, 1, denote degrees of precipitation; tr, trace; 0, no precipitate; controls, extract + saline, polysaccharide + saline.

The type XIV polysaccharide was allowed to diffuse in an Ouchterlony agar-gel plate[6] against *Ricinus* extract and horse anti-XIV serum. The precipitins present in the extract and in the serum gave a 'reaction of identity'[6]; this means that the *Ricinus* precipitin and the specific precipitin for type XIV pneumococcus precipitate the same substance.

Thus there is evidence that the activity of *Ricinus communis* precipitin is directed towards the structural configuration of the type XIV pneumococcus polysaccharide. Because it was not possible to test a complete series of more than seventy known types of pneumococcal polysaccharides, it cannot as yet be claimed that the *Ricinus communis* precipitin, in this system, is exclusively specific for type XIV; it is anticipated, however, that this will prove to be so, and that *Ricinus communis* seed extract will specifically agglutinate suspensions of type XIV pneumococci. I intend to complete this study by testing all known serological types of pneumococcus and purified preparations of their specific polysaccharides. The difficulty in obtaining all these materials is such that the project will take much time; it is therefore considered useful briefly to direct attention, at the present stage, to the relationship of the *Ricinus communis* precipitin to specific antibody to type XIV pneumococcus.

I am indebted to the State of New York Department of Health (Division of Laboratories and Research), Albany, New York, for the pneumococcal polysaccharides and horse anti-XIV serum.

G. W. G. Bird

Armed Forces Medical College,
Poona.

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Enzymatic Synthesis of Formylglutamic Acid by a Plant Protein

Silverman *et al.*[1], working with refined enzyme systems from hog liver, found indications that folinic acid functions directly only in the formylation of glutamate, with N-formylglutamic acid and tetrahydrofolic acid as the products. This communication reports the occurrence of a similar transformation in cell-free systems prepared from plant tissues.

Enzymatically active protein was obtained from mustard leaves. 2·5 kgm. of plant tissue were homogenized in an ice-cold solution of acetone and phosphate buffer *p*H 7·2 (v/v) in a Waring blendor. The solids were collected by filtration. Acetone was removed under vacuum and the residue extracted with 500 ml. of 0·02 *M* phosphate buffer *p*H 7·2. The precipitate which formed during the reduction of the volume to 250 ml. by pervaporation was removed by centrifugation and discarded. 7 gm. of ammonium sulphate were added per 100 ml. of the cell-free suspension (10 per cent of saturation at 0° C.). The precipitate was discarded. The supernatant received 14 gm. ammonium sulphate per 100 ml. (20 per cent of saturation). This precipitate was also discarded. The precipitate resulting from the treatment with 21 gm. ammonium sulphate per 100 ml. solution (30 per cent of saturation) was retained as the crude enzyme preparation. This crude product was suspended in water and dialysed for 24 hr. against 20 vol. 0·02 *M* phosphate buffer at *p*H 7·2.

Each biosynthesis medium contained 3·4 mgm. of the refined protein fraction; 0·2 μmole each of synthetic folinic acid, adenosine triphosphate, magnesium chloride, triphosphopyridine nucleotide, and a potential formyl group acceptor in buffer to a total volume of 3 ml. In addition to glutamic acid, glycine was used for comparison purposes. After incubation at 30° C. for 4 hr., 1 ml. was withdrawn and added to 1 ml. of 4 per cent perchloric acid. The resulting precipitate was removed and the supernatant was allowed to stand at room temperature for 30 min. to allow conversion of unused folinic acid to anhydrofolinic. The latter was determined spectrophotometrically at 360 mμ. The amount of folinic acid utilized was calculated by taking the difference in absorption between biosynthesis and a control containing denatured protein. This difference was referred to a standard curve prepared with synthetic folinic acid, since the optical density of anhydrofolinic at 360 mμ was found to be a linear function of folinic acid concentration. The results are presented in Table 1.

Table 1. Transformylase Activity of a Plant Protein Fraction in Presence of Synthetic Folinic Acid
(3·4 mgm. protein and 0·2 μmole folinic acid in 3 ml. biosynthesis medium*)

Tests	Acceptors	
	Glycine 20 μ*M*	Glutamate 4·0 μ*M*
Folinic acid transformed (μgm.)	1·5	7·8
Chromotropic acid:		
Biosynthesis	−	+
Control	−	−
Ninhydrin:		
Before hydrolysis—		
Biosynthesis	+	−
Control	+	+
After hydrolysis—		
Biosynthesis	+	+
Control	+	+

* For composition see text.

Separation of the biosynthesis product from the mixture containing glutamate by existing methods resulted in the isolation of a compound which was bound by chromotropic acid, under testing conditions similar to those described by Frisell *et al.*[2], and which reacted negatively with ninhydrin. After mild acid hydrolysis, the product was ninhydrin positive and formed a benzoyl derivative which melted at 135–137° C. (lit. for glutamic acid, 138° C.). Comparison indicated that the biosynthesized product was identical with a synthetic sample of formylglutamic acid. Glycine treated similarly did not become ninhydrin-negative and the product was not bound by chromotropic acid. However, the amount of unreacted folinic acid in each case diminished, indicating deformylation activity. These reactions were reversible

in the absence of potential formyl group acceptors and in the presence of formaldehyde and tetrahydrofolic acid. Results obtained by separate experiments show that glycine is converted into serine by this protein fraction in the presence of synthetic folinic acid. This is in substantial agreement with Wilkinson and Davies[3], who reported that tetrahydrofolic acid appears to be a requirement for serine-glycine interconversion in plant tissues. Thus, at least two types of formylation activity are demonstrated by these results; one in which the amino-group of glutamic acid is acceptor and the other in which the 2-C of glycine is acceptor.

Further purification of the protein fraction should make it possible to obtain more information about the specificities of the transformylases occurring in plant tissues, and thus permit a more detailed study of the possible role of formylglutamic in C-1 metabolism.

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ERNEST H. BYERS, JUN.
T. J. BOND

Department of Chemistry,
Baylor University,
Waco, Texas.

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Crystallization of a Photosynthetic Reductase from a Green Plant

WE would like to describe a method for crystallizing the protein now known as photosynthetic triphosphopyridine nucleotide reductase[1], formerly known as the methæmoglobin reducing factor for chloroplasts[2].

The reductase was prepared from frozen parsley (*Petroselenium sativum*) leaflets in May, following the procedure of San Pietro up to the preparation of an extract from the acetone precipitate. 4 kgm. parsley were used. After removing suspended matter the clear fluid, *p*H 8 in 0·005 *M tris* buffer, was chromatographed on a column of Whatman anion exchanger powder *DE* 50, 3·3 cm. diam. × 6·5 cm. This gave a narrow brown band at the top of the column, while yellow colour passed through. The column was washed with *tris* buffer (0·05 *M*, *p*H 8), followed by 0·2 *M* sodium acetate buffer, *p*H 5·5, until the washings were colourless and free from protein. The brown protein was then completely eluted with *M* acetate buffer, *p*H 5·5, giving 100 ml. of coloured solution. This was dialysed against 0·005 *M tris* buffer, *p*H 8, at 0° C. with stirring for 2 hr. and the 180 ml. solution passed through a second *DE* 50 column, 1 cm. × 3 cm. The column was washed with 0·05 *M tris* buffer, *p*H 8, followed in turn by 0·2 *M*, 0·5 *M* and 0·8 *M* acetate buffers, all at *p*H 5·5. The brown protein then occupied nearly all the column. Elution was carried out as before with *M* acetate buffer *p*H 5·5, giving 13 ml. of dark red-brown solution. This was then dialysed against half-saturated ammonium sulphate solution buffered with 100 ml. 0·33 *M* phosphate buffer at *p*H 6·7 (2 litres total volume) overnight at 0° C. The more concentrated solution, now 5·5 ml., was clarified in a centrifuge. The clear fluid was then treated with 0·15 ml. 5 per cent v/v acetic acid, when a faint turbidity appeared. After standing at 0° C. for 10 min. a precipitate was formed, consisting of minute spherical hedgehog crystal aggregates which later could be spun down in the centrifuge to a volume of about 0·5 ml. The crystal pellet was dark pinkish-brown and the liquid nearly colourless. Examination of the freshly formed crystal mass showed the crystals to be coloured, with virtually no accompanying insoluble material. This preparation was not suitable for photography as the crystals radiated in three dimensions. Crystals were therefore allowed to form on a slide under a cover glass, giving flat rosettes otherwise identical in appearance with those formed in the bulk of the solution. The crystals appeared to be plate-like as in Fig. 1*a* and more or less elongated as in Fig. 1*b*. The crystallization was repeated by dissolving the first pellet in 0·8 ml. disodium phosphate (0·05 *M*). 1 ml. saturated ammonium sulphate solution was then added and the solution spun. A small, pale precipitate was rejected. 5 per cent acetic acid was added as before to the clear fluid. Now the rosettes were mainly replaced by free crystal forms; minute elongated plates (10μ), thin plates and fine needles up to 150μ long were observed.

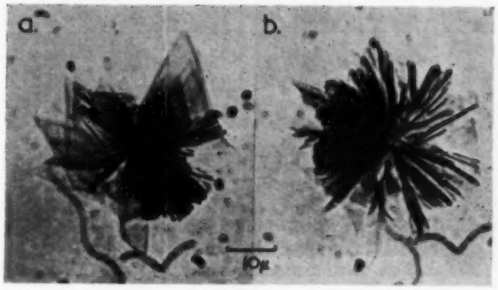

Fig. 1

This protein may be said to occupy a key position in the observed reducing properties of illuminated chloroplasts[3].

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R. HILL
FAY BENDALL

Department of Biochemistry,
University of Cambridge.

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PHYSIOLOGY

Differential Reactivity of Saliva from 'Tasters' and 'Non-Tasters' of 6-*n*-Propylthiouracil

IN a previous publication[1] a difference in reactivity between the saliva of 'tasters' and 'non-tasters' towards 6-*n*-propylthiouracil was reported. This communication attempts further to describe the above phenomenon and to relate the data thus obtained to the mechanism of taste in general and to taste-blindness in particular.

A 20-min. sample of mixed morning saliva stimulated by rubber band was centrifuged at +4° C. for

Table 1

No.	Compound	'△ optical density 8-min.' effect at $\lambda_{max.}$	△ optical density at molecular concentration of compound	Taste blind differentiator	Taste quality at $1{\cdot}5 \times 10^{-3}$ *M* concentration	Antithyroid activity
1	6-*n*-Propylthiouracil	+	$3{\cdot}75 \times 10^{-5}$	+	bitter	+
2	2-Mercapto-imidazole	+	$3{\cdot}75 \times 10^{-5}$	+	bitter	+
3	1-Methyl-2-mercapto-imidazole	+	$3{\cdot}75 \times 10^{-5}$	+	bitter	+
4	2-Mercapto-5-carbomethoxy imidazole	−	$3{\cdot}75 \times 10^{-5}$	−	bitter	−
5	2-Mercapto-4 (or 5)-imidazole carboxylic acid	+	$3{\cdot}75 \times 10^{-5}$	−	sour	−
6	1-Methyl-2-mercapto-5-imidazole carboxylic acid	+	$3{\cdot}75 \times 10^{-5}$	−	bitter	−
7	Creatinine	−	$1{\cdot}5 \times 10^{-4}$	−	bitter*	+
8	Imidazole	−	$6{\cdot}0 \times 10^{-4}$	−	bitter	?
9	Quinine	−	$1{\cdot}5 \times 10^{-4}$	−	bitter	−

* Bitter taste quality only from 16–32-fold or higher concentration.

30 min. at 3,000 r.p.m. The supernatant was re-centrifuged and 1-ml. portions of it were diluted 1 : 5 with *tris* buffer to *p*H 7·36 at 23° C. and supplemented with 6-*n*-propylthiouracil; final concentration of *tris* and 6-*n*-propylthiouracil are 0·05 *M* and $3{\cdot}75 \times 10^{-5}$ *M*. Later, the rate of reaction in a sample was followed in a Perkin-Elmer 'Spectracord 4000-*A*' spectrophotometer at 17°, 27° and 37° C. in the ultra-violet range. The decrease in optical density was read at the λ (maximum) between 276 and 284 mμ in 8 min. This decrease, which is not photosensitive, indicates a significantly faster rate of reaction for extreme 'non-tasters' than for 'tasters' (Fig. 1).

The '△ optical density 8-minute' effect can be simulated in the absence of saliva by the action of hydrogen peroxide on 6-*n*-propylthiouracil. The decrease in optical density thus produced, however, increases with increasing temperature, which effect is the converse of that obtained with saliva.

The *p*H dependence of the reactivity ('△ optical density 8-minute' effect) of saliva with 6-*n*-propylthiouracil is depicted in Fig. 2.

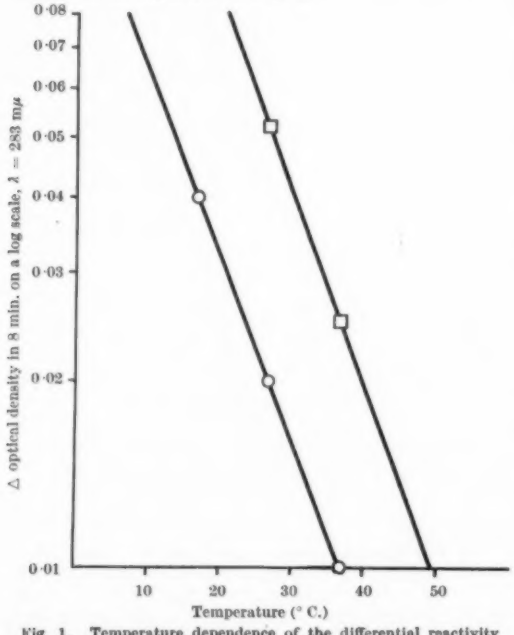

Fig. 1. Temperature dependence of the differential reactivity ('△ optical density 8-min.' effect) of the saliva of a typical 'taster' and typical 'extreme non-taster' toward 6-*n*-propylthiouracil from a population of 32 subjects. ○ and □ represent results obtained from salivas of donors with taste-thresholds of $4{\cdot}7 \times 10^{-5}$ *M* and $6{\cdot}0 \times 10^{-3}$ *M* 6-*n*-propylthiouracil respectively

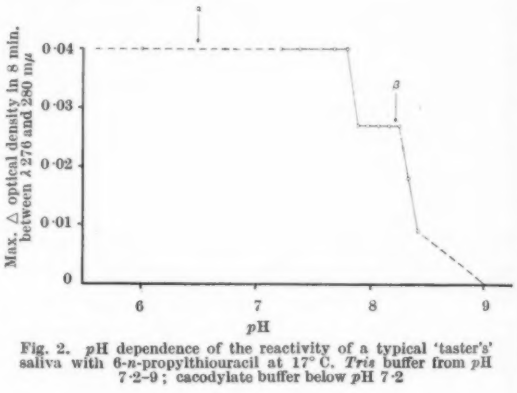

Fig. 2. *p*H dependence of the reactivity of a typical 'taster's' saliva with 6-*n*-propylthiouracil at 17° C. *Tris* buffer from *p*H 7·2–9; cacodylate buffer below *p*H 7·2

The sites α and β on the slopes of the curve approximate the *pK*(OH) values of the mono- and diiodo-derivatives of L-tyrosine. These thyroxine precursors, in fact, do lower at least 4–8- or even 32-fold the taste threshold of a subject towards 6-*n*-propylthiouracil if administered simultaneously. Specifically, 3×10^{-3} *M* 3-monoiodotyrosine and $7{\cdot}5 \times 10^{-4}$ *M* tyrosine or 3,5-diiodotyrosine have been used as solvents as well as placebos in the Harris and Kalmus[3] procedure. The actual originally defined 6-*n*-propylthiouracil threshold of the subject was a determining factor of the ability of monoiodotyrosine and/or diiodotyrosine to increase taste acuity towards 6-*n*-propylthiouracil. (The original 6-*n*-propylthiouracil threshold can also be altered by simply raising the temperature of the solution of the substance to 40° C.) Taste acuity of tasters with a $3{\cdot}75 \times 10^{-4}$ *M* 6-*n*-propylthiouracil threshold was increased with any of the three thyroxine precursors.

A similar increase in taste acuity towards quinine was produced under identical conditions. Only diiodotyrosine was capable of lowering thresholds of $1{\cdot}5 \times 10^{-3}$ *M* concentration of 6-*n*-propylthiouracil. None of the precursors was effective at threshold $6{\cdot}0 \times 10^{-3}$ *M* of 6-*n*-propylthiouracil, that of 'extreme non-tasters'.

Among the bitter-tasting anti-thyroid compounds which contain the thiocarbamide grouping, taste-blind differentiators other than 6-*n*-propylthiouracil also exhibit the '△ optical density 8-minute' effect. This effect alone, however, does not convey the quality of a taste-blind differentiator to a compound of the thiocarbamide type. Compounds No. 5 and 6 in Table 1 exemplify our contention. Another more tentative conclusion can be drawn from Fig. 1 in the light of additional (unpublished) results: anti-

thyroid compounds of the thiocarbamide type with no taste-blind differentiating quality, such as creatinine (synthesized via creatine (*Org. Synthesis*, **4**, 15; 1925); such a creatinine is free of picric acid, which is present in commercially available preparations. No. 7 in Table 1), an isosterically substituted thiocarbamide, are likely to produce their anti-thyroid activity[2] through another mechanism than that characteristic for compounds No. 1, 2 and 3.

Biochemical, genetic, pathophysiological and psychological aspects of the part played by thyroxine and its precursors in the mechanism of taste and 'taste-blindness' are under study and will be published elsewhere.

This work was carried out with the technical assistance of Edwin L. Mead. The active interest and helpful suggestions of Drs. B. Pasamanick and F. A. Kruger, Columbus, and M. D. Armstrong, Yellow Springs, are thankfully acknowledged. Our special thanks are due to Dr. S. Dinitz, Columbus, the first subject whose saliva elicited the 'Δ optical density 8-minute' effect, and to Drs. E. C. Kornfeld, R. G. Jones and W. R. Kirtley, from the Eli Lilly and Co., Indianapolis, who courteously supplied us with the imidazole derivatives.

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FRANCES GRIFFIN
ROLAND FISCHER

Research Division,
Columbus Psychiatric Institute
and Hospital, and
Department of Physiological Chemistry,
Ohio State University College of Medicine,
Columbus 10, Ohio. March 17.

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Flight by Man-Power

BEING interested in the possibility of flight by man-power, we have read Dr. D. R. Wilkie's communication[1] on the "Work Output of Animals: Flight by Birds and by Man-power".

We agree[2] that for mechanical similarity the power requirement is a function of (weight)$^{1.167}$. Moreover, for flying insects and birds[3] we obtained from Pütter's data a different exponent for this statistical relationship, namely (weight)$^{0.667}$. It is interesting to note that according to the theory of biological similarity[2], the limits for these exponents of body-weight are 1·167 for mechanical similarity, and 0·667 for the electrodynamical similarity, whereas for biological similarities, power is a statistical function of (weight)$^{0.72}$.

Thus, in accordance with the principle of mechanical similarity the power requirement for a flying machine is proportional to (weight)$^{1.167}$; the same relationship was obtained by Nonweiler (quoted in Dr. Wilkie's paper) based on purely aerodynamic considerations. This equivalent conclusion provides evidence that the power necessary to initiate flight exclusively by muscular effort, without additional external forces (potential energy, wind velocity, etc.), is a function of (weight)$^{1.167}$. The slope of this theoretical relationship (line *E* of Fig. 1 in Dr. Wilkie's paper) intersects line *C* of the same graph, which represents the steady-state maximal effort that man is able to perform in oxygen balance for at least 5–30 min.

The theory of biological similarities[2] states that all power functions of living beings are related to (weight)$^{0.72}$, and since it was found[3] that flying insects and birds have mean power requirements which are a statistical function of (weight)$^{0.667}$, we presume that this represents the optimal conditions of natural flight which man should try to achieve during artificial flight.

Furthermore, from the experimental data of Bjerknes and of Müllenhoff, we have calculated[3], by extrapolation from the regression equation based on data for a weight range from the fly to the stork, that a man-powered flying machine (70 kgm. man and 30 kgm. flight-accessories) should have approximately the following characteristics for slow flapping flight: (*a*) flight surface, 6 m.^{2}; (*b*) pressure, 16·8 kgm./m.^{2}; (*c*) speed, 18·2 m./sec.; (*d*) power, 6 kgm.-m./sec.; (*e*) force of the wing-beat, 19·3 kgm.-weight; (*f*) speed of the wing tip, 5·8 m./sec.; (*g*) length of the wing, 3·15 m.; (*h*) wing-beats per second, 0·592/sec.

Another aspect of the same problem is related to the different density of birds and men. In order to have similar flight conditions, it might be wise to construct a man-powered flying machine of approximately the same density of those of insects and birds, so that the aerodynamics of the whole machinery should be similar to the natural models. The transfer of muscular power to the wings and the sensory information of the actual flight conditions must be based on the flight-physiology of insects and birds, since the reflex regulation of flight is of paramount importance.

We agree with Dr. Wilkie in the sense that man-powered flight is possible and should be tested experimentally, because new hypotheses and materials (both metals and plastics) are available which can be used to solve some of the engineering problems involved.

E. GUERRA
B. GÜNTHER

Departamento de Ciencias,
Universidad de Chile,
Casilla 130-V,
Valparaíso, Chile.

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Effect of Nitrate Ion on the Distribution of Sucrose in the Rat's Ventricle

CURRENT work in this laboratory shows that the exposure of driven rat ventricle strips to Locke's solutions, in which all the chloride has been substituted by nitrate, results in an increase of the total sodium in the tissue. In order to determine how this increase was partitioned between the extracellular and the intracellular compartments we attempted to measure the extracellular space by the distribution of sucrose. The results presented below show that this cannot be done in the case of nitrate, and reveal an interesting feature of the action of this anion, which may be that of increasing the permeability of cardiac tissue to a molecular species not ordinarily considered as being able to traverse the cell membrane.

Table 1. Water Content and Sucrose Space in Driven Ventricle Strips

Perfusion time	Total water content (percentage of wet weight)		Sucrose space (percentage of wet weight)		Sucrose space (percentage of total water)	
	Cl	NO_3	Cl	NO_3	Cl	NO_3
5 min.	(10) 75·56	(7) 77·41	22·23	34·18	29·43	44·14
15 ,,	(10) 75·71	(5) 77·54	27·44	40·03	36·23	51·62
30 ,,	(12) 75·63	(8) 77·18	28·69	48·51	37·91	63·08
1 hr.	(12) 75·31	(6) 77·90	30·88	49·95	41·03	64·11
3 ,,	(4) 75·88	(5) 77·84	34·90	71·13	46·01	91·42
5 ,,	(4) 75·90	(5) 78·25	53·60	76·53	70·61	97·80
7 ,,	(5) 75·42	—	62·14	—	82·38	—
9 ,,	(3) 75·50	—	63·04	—	83·50	—

The preparation of the tissue[1], the composition of the anionically substituted Locke's solution[2], and the experimental procedure[3,4] have all been described previously. In the present experiments the electrically driven ventricle strips were maintained at 37° C. in either chloride- or nitrate-reference solutions for periods up to 9 hr. in the former case and 5 hr. in the latter. Both types of solution contained sucrose in a concentration of 600 mgm./100 ml. The ventricle strips were removed from the muscle baths at the time intervals indicated in Table 1, dried and analysed for sucrose by the method of Ross and Mokotoff[5]. Since nitrate was found to interfere with the colorimetric determination of sucrose it was necessary to eliminate this anion by passing the solution through an anion exchange resin, 'Duolite *A*-101*D*'. Both wet and dry weights were recorded to determine water content.

The results presented in Table 1 show a small but significant increase in the wet weights of the nitrate-treated muscles, amounting to about 2 per cent above those of the chloride controls. Fig. 1 shows the time-course of sucrose distribution in the two classes of muscle. It is evident from Fig. 1 that the time-course of sucrose distribution can be separated, as has been done by Creese[6], into several kinetic components: an early linear phase, 1, an early exponential phase, 2, and a late exponential phase, 3. It is risky, of course, to reify kinetics into anatomical compartments, but it is significant that the early exponential phase becomes asymptotic to a line parallel to the X-axis which cuts the Y-axis at roughly the value of the extracellular phase[7,8] in the case of chloride. On that basis, the delayed exponential phase may represent either the rate of adsorption of sucrose by some structural barrier or, more probably, its slow penetration into the cell, which can be shown by extrapolation to begin very early in the process of sucrose uptake by the muscle. Treating all the phases, on a first approximation, by means of first-order kinetics, the following values were obtained for the three velocity constants:

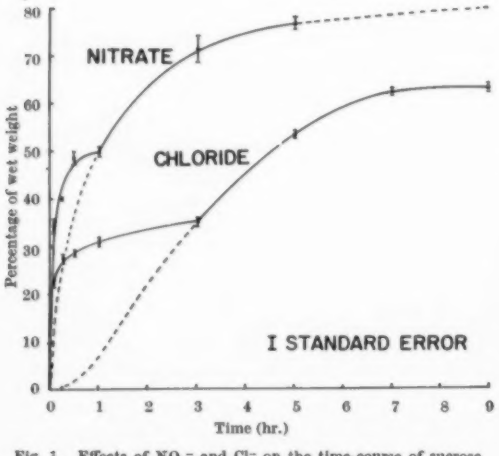

Fig. 1. Effects of NO_3^- and Cl^- on the time-course of sucrose distribution in the driven ventricle

	k_1/min.	k_2/min.	k_3/min.
Cl	0·245	0·0268	0·0046
NO_3	0·662	0·0643	0·0118
NO_3/Cl	2·70	2·25	2·59

Not only is the velocity of the several processes greater in nitrate than in chloride but, in addition, the apparent volume of distribution of sucrose is also increased by this anion, becoming commensurate with that of total tissue water in about 5 hr.

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S. T. Taketa
C. G. Alexander
G. A. Feigen

Department of Physiology,
Stanford University School of Medicine,
Stanford, California.

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An Unidentified Pharmacologically Active Substance extracted from Cerebral Hemispheres

While estimating the 5-hydroxytryptamine content of rat brain, it was observed that the acetone extract stimulated the isolated rat uterus even in the presence of atropine and 2-bromo-lysergic acid diethylamide (*BOL*–148, 10^{-7} gm./ml.). Different parts of the rat brain were then separately extracted and examined, and by far the largest proportion of the active material was found in the cerebral hemispheres. Similar results were obtained when extracts of different areas of other mammalian brains were tested. A study was therefore made of the properties of this smooth-muscle-stimulating substance present in extracts of cerebral hemispheres of mammals.

Smooth-muscle-stimulating substance stimulates the isolated atropinized rat uterus after a latent period of about 45 sec. and continues to increase its tone over the next 5 min. The threshold dose for this effect is, in terms of fresh cerebral hemisphere, about 50 mgm. for the rat and rabbit and about 100 mgm. for the dog. In slightly higher doses, smooth-muscle-stimulating substance also stimulates the isolated atropinized rat fundus, rat colon and rat duodenum. It produces a contraction of the isolated guinea pig ileum in the presence of atropine, mepyramine and *BOL*–148, but the most sensitive preparation used so far is the rabbit duodenum, which is stimulated by an extract of only 20 mgm. of the rat, rabbit or dog

cerebral hemisphere. On the blood pressure of the cat or rabbit, smooth-muscle-stimulating substance exerts a depressor response both before and after atropine and mepyramine, yet it is pressor on the blood pressure of the rat. On cardiac and skeletal muscle, it is relatively inactive.

The active material is extracted from cerebral hemispheres with different concentrations of acetone (1–20 vol.) and with trichloracetic acid (2 vol. of 10 per cent) but not with saline containing 0·1 *N* hydrochloric acid. Extracts prepared in this way retain activity for more than 1 month if maintained at 0° C. at neutral or acid *p*H values; but the activity slowly disappears when the extract is kept at *p*H 11. Smooth-muscle-stimulating substance is not inactivated by incubation with trypsin or chymotrypsin. On paper chromatograms, the active material has an R_F value of 0·60 in a sodium chloride solvent (8 per cent w/v), but it does not move from the origin in solvents of butanol–acetic acid, *iso*propanol–ammonia, or aqueous methanol (60 per cent v/v). With potassium iodate, ninhydrin reagent, Pauly's reagent, Folin–Ciocalteau's phenol reagent, or Ehrlich's reagent, the active material gives no colour reactions characteristic of catechols, amines, amino-acids, compounds containing hydroxyl groups, or indoles.

Thus, smooth-muscle-stimulating substance differs from histamine (which is inactive on the rat uterus), 5-hydroxytryptamine (actions blocked by *BOL*–148), acetylcholine (blocked by atropine), bradykinin (insoluble in acetone)[1], substance *P* (inactivated by chymotrypsin)[2], irin (inactive on rat uterus)[3], γ-aminobutyric acid (inactive on rabbit duodenum)[4], γ-aminobutyryl choline (blocked by atropine)[5], nasal mucosa factor (unstable in acid)[6], and cerebellar factor (which is insoluble in acetone)[7]. It is unlikely to be a polypeptide.

P. F. L. Andrews
Shirley A. P. Price
G. B. West

Department of Pharmacology,
School of Pharmacy,
University of London,
29–39 Brunswick Square,
London, W.C.1. March 23.

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Production of Muscular Weakness in Rats by a Creatine Analogue

In an effort to clarify the role of abnormal creatine metabolism in producing muscular dystrophy a search for an antimetabolite to creatine has been made. If a specific defect in either the synthesis or utilization of creatine could be produced by an antimetabolite, it would then be possible to assess the change in muscle structure and metabolism which results from impaired creatine metabolism.

One analogue of creatine which has been tested is D,L α-guanidinopropionic acid (alacreatine)[1]. A group of 10 weanling Sprague–Dawley rats of both sexes was fed a purified diet containing 18 per cent casein, 74·4 per cent sucrose, 3 per cent hydrogenated vegetable fat, 2 per cent cod liver oil, and the usual salts and vitamins and supplemented with 2 per cent alacreatine. While receiving this diet during a 6-week period the rats gradually became quite weak in comparison with control animals receiving only the purified diet. The type of weakness seen in the rats receiving alacreatine resembled that seen in rabbits suffering from nutritional muscular dystrophy resulting from vitamin E deficiency. When placed on their side they had difficulty in righting themselves, particularly after exercise.

The finding of severe muscular weakness in animals fed an analogue of creatine strongly suggests that the analogue is a metabolic antagonist to creatine and that the weakness of muscular dystrophy is the result of the abnormal creatine metabolism of that disease. Study of the enzymes of creatine synthesis (transamidinase and guanidoacetate methylpherase) and utilization (creatine kinase) in animals given alacreatine should readily demonstrate a specific defect in creatine metabolism if one is present. This work and experiments to determine the influence of creatine feeding on the syndrome are now in progress.

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Coy D. Fitch
James S. Dinning

Department of Biochemistry,
University of Arkansas,
School of Medicine,
Little Rock,
Arkansas.

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Damage to Liver induced by DL-Methionine-Ethylester

Perri *et al.* have observed that tryptophan-methylester inhibits the growth of several bacteria[1]. Horváth *et al.* found that the adaptive enzyme product of *Penicillium chrysogenum* inhibits glycine-, *iso*leucine- and phenylalanine-methylester and supposed that the amino-acid-ester takes part in protein synthesis after the acyl-activation and with its bound carboxyl group stops peptide production[2]. According to this hypothesis the amino-group of the esterified amino-acid is more reactive than the natural one, therefore the activated amino-acid reacts mainly with the ester. Although in the above-mentioned work no completion experiments were performed, the results suggest that the amino-acid-esters behave like antimetabolites on lower organisms.

On the basis of the above we supposed that DL-methionine-ethylester is a methionine antagonist causing liver injury, which then could be inhibited by methionine administration.

Dimethionine-ethylester was synthesized by esterification of DL-methionine and of absolute ethanol in dry hydrochloric acid. The salt of the compound was isolated and recrystallized from absolute ethanol. The dimethionine-ethylester thus produced was chromatographically uniform.

Albino rats of both sexes weighing 80–90 gm. were used in the experiments. They were fed a normal diet during the experimental period and were divided in three groups. 7 rats (Group I) were given 0·34 m.mole methionine; 16 rats (Group II) 0·24 m.mole dimethionine-ethylester and 7 rats (Group III) 0·34 m.mole methionine + 0·24 m.mole dimethionine-

ethylester daily for 17 days, intraperitoneally. The dimethionine-ethylester was always administered after neutralization. Six rats from Group II died spontaneously between the fifth and the seventeenth day; in the other groups no death occurred. On the eighteenth day the rats were killed by decapitation, the liver was immediately removed, weighed and worked up histologically.

All the animals in group II had enlarged livers; in Groups I and III the livers were of normal size (Table 1).

Table 1

Group	No. of rats	Daily intraperitoneal administration in m.mole/rat	Days of administration	Liver-weights in gm./100 gm. body-weight
I	7	0·34 methionine	17	3·94 ±0·42
II	16	0·24 methionine-ethylester	17	4·5 ±0·41
III	7	0·34 methionine + 0·24 methionine-ethylester	17	3·62 ±0·45

$t = 1·06$, $P > 0·2$

Histological examination revealed that in Group II only one liver was normal; the others showed parenchymatous degeneration combined with perilobular fatty infiltration of various grades. Among the seven rats of Group III five had normal livers, and two showed mild fatty infiltration (Figs. 1, 2 and 3).

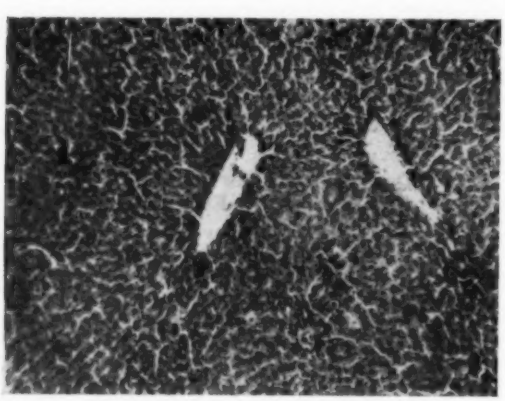

Fig. 1. Liver from group I. Normal structure. Hæmatoxylin-eosin stain. (× 200)

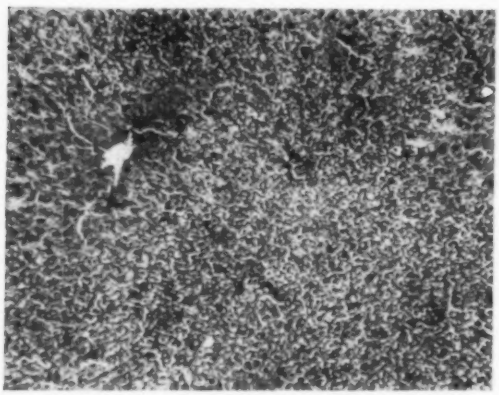

Fig. 2. Liver from group II. Parenchymatous degeneration with severe perilobular fatty infiltration. Hæmatoxylin-eosin stain. (× 200)

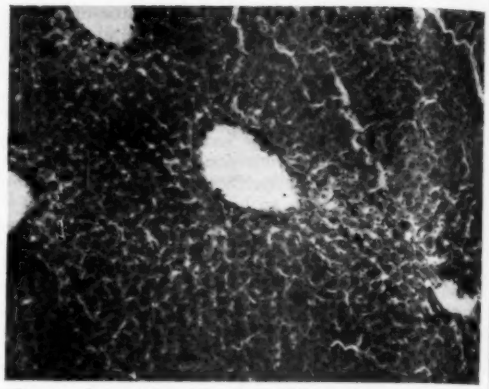

Fig. 3. Liver from group III. Mildly swollen liver baulks. Hæmatoxylin-eosin stain. (× 200)

It is therefore concluded that dimethionine-ethylester induces enlargement and pathological changes in the liver. The enlargement is presumably due to an increase in fat and water contents, while the injury itself depends on the individually different activity of the non-specific esterase. In this way various quantities of dimethionine-ethylester were destroyed, resulting in injury of varying gravity. This assumption, however, will have to be supported by further investigations.

I. Fehér
F. V. Selmeczi
K. Szalay
T. Kemény

Institute of Pathophysiology,
University Medical School,
Budapest.

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Effect of Some Aromatic Hydroxy Compounds on the Phagocytic Activity of the Reticulo-Endothelial System

It has been reported previously that œstrogens stimulate the reticulo-endothelial macrophages in the spleen, liver and lymph nodes and lead to mobilization of the macrophages into the blood-stream and thus probably to an increase in the general defence of the body against infection and perhaps new growths; and that these results suggested the desirability of finding a substance which possesses a still more powerful stimulating effect on the reticulo-endothelial system without the ill effects of œstrogens[1]. Since then, the effects of various stilbenes, steroids, aliphatic hydrocarbons, antibiotics and sulphonamides on the phagocytic activity of the reticulo-endothelial system have been investigated and reported[2–6]. So far, the strongest stimulants of phagocytic activity are also strongly œstrogenic and it would appear that the configuration of the molecule which confers œstrogenic activity is also essential for reticulo-endothelial stimulation. Many other compounds are therefore being examined in the hope that the results may throw more light on the nature of the stimulative factor.

The present communication is a report on the effect of four aromatic hydroxy compounds on the phagocytic activity of the reticulo-endothelial system.

Thirty male white mice (*T.O.* Swiss strain) of 20–27 gm. body-weight were used in these experiments. Five animals were used to test each substance, each animal receiving one subcutaneous dose of 0·5 mgm. of the compound in 0·05 ml. arachis oil daily for 6 days. On the eighth day the phagocytic activity was measured by the rate of disappearance of a known amount of specially prepared carbon from the circulating blood[7], the procedure used being as follows. Each animal was given one intravenous injection of carbon of particle size 250 Å., the dose of the carbon being calculated on the basis of 16 mgm. per 100 gm. body-weight. Blood was then taken at short intervals during the first 60 min. following the injection, and the concentration of the carbon in each blood sample was measured by an absorptiometer. The logarithmic values of the absorptiometer readings lie along a straight line when plotted against time, and the slope of the line has been taken as the measure of total body phagocytic activity or the phagocytic index, which is denoted in the present communication by the symbol *K*.

Ten of the animals were used as controls and received 0·05 ml. arachis oil once daily for 6 days. The phagocytic activity was then measured on the eighth day by the carbon method referred to above. The arachis oil controls showed a phagocytic index or *K* value of 13 ± 2·4.

Table 1. Effect of Some Aromatic Hydroxy Compounds on the Phagocytic Activity of the Reticulo-endothelial System

Compound used	Phagocytic index (*K* value)
Racemic 3 : 4-di-*p*-hydroxyphenylhexan-1 : 6-diol	16 ± 1·6
meso 3 : 4-di-*p*-hydroxyphenylhexan-1 : 6-diol	17 ± 2·6
meso $\beta\beta'$-di-*p*-hydroxyphenyladipic acid	18 ± 3·3
1 : 2-Di-α-naphthyl-1 : 2-dihydroxyacenaphthene	15 ± 1·8
Arachis oil controls	13 ± 2·4

The results are shown in Table 1. Compared with the control values, it can be seen that the four compounds investigated, which are similar in type, have little or no effect on the phagocytic activity of the reticulo-endothelial system.

We are indebted to Sir Charles Dodds for generously supplying these compounds. We also gratefully acknowledge assistance from the Central Research Fund of the University of London and from the Medical Research Council.

T. Nicol
C. Druce
C. C. Ware

Department of Anatomy,
King's College,
London, W.C.2.
March 1.

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PATHOLOGY

Production of Tumours in *Rattus* (*Mastomys*) *natalensis* by Polyoma Virus

Rattus (*Mastomys*) *natalensis*, the multi-mammate mouse, is an African rodent intermediate in size between the rat and the mouse. These animals have been used in microbiological studies because they are highly susceptible to infection by plague bacilli[1], *Schistosoma mansoni*[2], and *Borrelia duttonii*[3]. They have recently been used in cancer research laboratories following Oettle's report of a high incidence of spontaneous adenocarcinoma of the stomach in a *Mastomys* colony in Johannesburg, South Africa[4]. Since the polyoma virus has been shown to produce tumours in mice, Syrian hamsters, rats and rabbits[5], we were interested in determining the response of *Mastomys* to this agent.

A small colony of *Mastomys* is now maintained by Dr. H. L. Stewart and Dr. K. C. Snell in the Laboratory of Pathology of the National Cancer Institute in Bethesda, Maryland. Four pregnant *Mastomys* were obtained from this colony.

The strain of polyoma virus used was isolated originally from a filtrate of an extract of a murine parotid gland tumour, and the details of the derivation of this strain have been reported by Dawe and his associates[6]. The virus has been serially passaged in cultures of milk-adapted murine lymphoma cells (strain *P*388*D*1)[7], and fifth, tenth and fourteenth tissue culture passage pools were used to inoculate the *Mastomys*. Tissue culture medium composed of 20 per cent autoclaved fatless milk and 80 per cent mixture 199 was used to inoculate control animals.

New-born *Mastomys*, less than 12 hr. old, were inoculated subcutaneously on the back with 0·05–0·10 ml. of virus pool or control tissue culture medium. The size of the litters, type of inocula and number of animals studied at autopsy are presented in Table 1. Six months after inoculation, 24 of the 30 animals that had received virus were dead; however, because of cannibalism, it was not possible to autopsy 7 of these. Tumours were found in 14 of the 17 animals that were autopsied. As shown in Table 2, 13 *Mastomys* had tumours in the kidneys, 4 had tumours in the liver, 2 had tumours in the heart and 2 had tumours in the subcutaneous tissues. None of the animals inoculated with control tissue culture medium has died 6 months after inoculation and no tumours have been observed or palpated in these animals.

The tumours of the kidneys varied on gross examination from small nodules 0·2–0·3 cm. in diameter to masses measuring 3–5 cm. in greatest dimension, and they were frequently multi-focal and bilateral. Histologically, they were composed of ovoid and elongated cells which were sometimes

Table 1. New-born *Mastomys* inoculated with Polyoma Virus

Litter	No. of animals inoculated	Inoculum	Alive 6 months after inoculation	Dead: No autopsy	Dead: Autopsy
1	10	200,000 *TCD*50 of 14th *TC* passage	0	6	4
2	10	8,000 *TCD*50 of 10th *TC* passage	4	1	5
3	10	8,000,000 *TCD*50 of 5th *TC* passage	2	0	8
4	7	control fluid	7	0	0

Fig. 1. Renal sarcoma in *Mastomys* inoculated with polyoma virus. A renal tubule is surrounded by tumour cells. Hæmatoxylin and eosin. (× *c.* 416)

arranged in interlacing bundles, and all were diagnosed as sarcomas. The nuclei of the tumour cells were pleomorphic and hyperchromatic, and frequently there was clumping of chromatin along the nuclear membranes with central clear areas in the nuclei. Similar nuclear changes have been observed in cells of the *P*388*D*1 strain in tissue culture after infection with polyoma virus[7]. Occasionally, the tumours were very vascular and the neoplastic cells were arranged around blood vessels in a manner similar to that described in the intracranial sarcomas produced by polyoma virus in the Syrian hamster[8].

The tumours in the livers of 3 of the animals (Nos. 10, 12, 13, Table 2) were angiomatous in type and similar to those described in the livers of Syrian hamsters inoculated with polyoma virus[9]. The 3 animals with these angiomatous tumours were found dead, and at autopsy they had hæmoperitoneum associated with rupture of vessels of the hepatic tumours.

In the liver of one *Mastomys* (No. 15, Table 2), a white nodule 0·3 centimetre in diameter was noted grossly on the cut surface of the median lobe just beneath the capsule. Histologically, this was composed of irregularly shaped duct-like structures lined by columnar cells with pleomorphic hyperchromatic nuclei and amphophilic cytoplasm. The tumour was not encapsulated, and appeared to invade adjacent liver parenchyma. It was diagnosed as an adenocarcinoma, and we believe that it probably originated from bile duct epithelium, although the possibility of origin from altered hepatic cells has not been eliminated. Tumours of this type have not been described in mice, rats, hamsters or rabbits inoculated with polyoma virus. Proliferation of bile ducts, however, has been described in the livers of *Mastomys* infected with *Schistosoma mansoni*[2]. The relationship of this tumour to the inoculation of polyoma virus is therefore not clear at present.

The two heart tumours were small, each measuring approximately 0·1 cm. in greatest diameter. One was found beneath the endocardium of the right ventricle adjacent to the pulmonic valve, and the other in the wall of the right atrium. Histologically, they were composed of fusiform and oval cells, and resembled the sarcomas found in the hearts of Syrian hamsters inoculated with polyoma virus[9]. The subcutaneous tumours in the *Mastomys* were also diagnosed as sarcomas and were histologically similar to the subcutaneous tumours produced by polyoma virus in Syrian hamsters[9].

It has been possible to isolate polyoma virus in tissue culture from a suspension of one of the renal sarcomas of the *Mastomys*. At the time of autopsy, a portion of the tumour of the kidney from animal No. 11 (Table 2) was frozen at − 20°. It was later thawed and a 20 per cent suspension of tumour tissue was made in tissue culture medium (20 per cent autoclaved fatless milk and 80 per cent mixture No. 199). Cultures of milk-adapted *P*388*D*1 cells were inoculated with 0·25 ml. of the 20 per cent tumour tissue suspension. In one of two inoculated tubes, a cytopathological effect similar to that seen in cultures inoculated with polyoma virus was observed after 21 days. The supernatant fluid from this culture was then inoculated into 9 new-born Syrian hamsters, and all died within 30 days. Five were autopsied and all had multiple tumours similar to those previously described in hamsters inoculated at birth with polyoma virus[9].

Although spontaneous tumours have been described in *Mastomys*, they have rarely been found in animals younger than one year of age, and they differ from the sarcomas of the kidneys and heart, and angiomatous tumours of the liver found in the animals inoculated with polyoma virus[4]. With the possible exception of the adenocarcinoma of the liver in one of our *Mastomys*, it therefore seems unlikely that the tumours we have observed are spontaneous, and we believe that they are related to the inoculation of the polyoma virus. The mechanism by which the virus

Table 2. Tumours in *Mastomys* inoculated with Polyoma Virus

Animal	Litter No.	Sex	Age at death	*K* = killed *F* = found dead	Tumours			
					Kidneys	Liver	Heart	Subcutis
1	1	F	61	*K*	+	−	−	−
2	1	F	101	*F*	+	−	−	−
3	1	M	104	*K*	+	−	+	−
4	1	F	160	*K*	+	−	−	+
5	2	M	150	*K*	−	−	−	−
6	2	F	150	*K*	−	−	−	+
7	2	M	159	*K*	−	−	−	−
8	2	F	165	*K*	−	−	−	−
9	2	M	172	*F*	+	−	−	−
10	3	F	36	*F*	+	+	−	−
11	3	M	45	*K*	+	−	+	−
12	3	M	46	*F*	+	+	−	−
13	3	M	52	*F*	+	+	−	−
14	3	F	134	*F*	+	−	eaten	−
15	3	M	151	*F*	+	+	−	−
16	3	F	156	*F*	+	−	−	−
17	3	F	159	*F*	+	−	−	−

produces tumours, however, remains completely obscure at present.

Since some difference of opinion exists whether to group the *Mastomys* taxonomically with the rat (genus *Rattus* Fischer) or with the mouse (genus *Mus* L.), the response of *Mastomys* to polyoma virus is of interest. The renal sarcomas produced by polyoma virus in *Mastomys* are grossly and histologically similar to tumours produced by polyoma virus in the kidneys of rats and mice[5]. The sarcomas in the heart, however, have not been described in either rats or mice, but are very frequently produced by polyoma virus in the Syrian hamster[6]. Thus far, no parotid gland tumours or thymic tumours similar to those produced by polyoma virus in mice have been found in the *Mastomys*. It should be noted, however, that the virus pools we have used show very little oncogenic activity in mice, although they readily produce tumours in Syrian hamsters.

Additional studies of *Mastomys* inoculated with varying doses and different strains of polyoma virus are now in progress in our laboratory and will be reported in detail later.

Alan S. Rabson
William J. Branigan
Frances Y. Legallais

Laboratory of Pathology,
National Cancer Institute,
Bethesda,
Maryland.

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Hæmodynamic Aspects of Atherogenesis

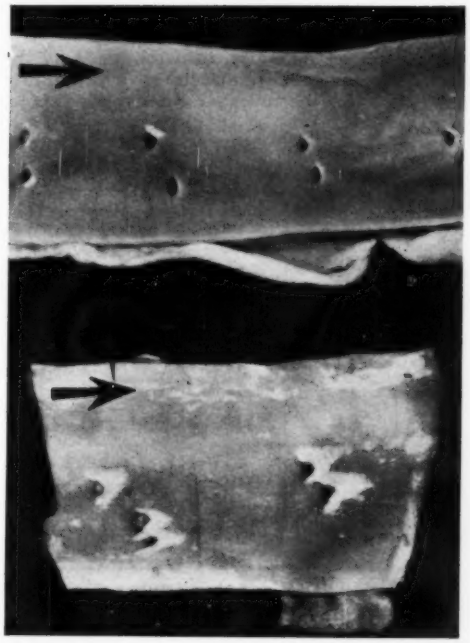
Atherosclerosis is generally regarded as a disease process which is mainly due to disorder of metabolic factors such as lipoproteins, hormones, heparin, etc. These factors have been extensively studied, especially with respect to lipoproteins. However, careful examination of the artery at autopsy shows that plaques are scattered all along an arterial branch. An artery of considerable length uniformly and concentrically coated with lipid material without localized regions of sclerosis seems to be quite rare. On the contrary, plaques are in the majority of the cases deposited in a peculiar pattern. Such deposition of lipid materials and the topographical arrangement of plaques cannot be explained readily by general metabolic factors alone. Thus a method of quantitative grading of atherosclerosis has been developed[1] in order to distinguish the role of the general metabolic factors and local factors. This technique has been applied to a study of the interrelationship between cerebral and coronary atherosclerosis[2]. By quantitative grading of sclerosis and systematic analysis of the coronary artery for relative position and severity of plaques one is able to demonstrate the significance of local factors in atherogenesis.

Table 1. Effect of Bend in an Artery on Atherogenesis

No. of sections	*I/E*	Position of sections
135	47·5 ± 14·2*	Pre-bend
135	59·8 ± 11·8	Bend
139	54·9 ± 13·7	Bend
139	48·4 ± 15·4	Post-bend
125	42·7 ± 16·4	Post-bend
105	32·9 ± 14·3	Post-bend

I, Area of intimal material; *E*, total area of arterial wall; *I/E*, atherosclerosis units.
* Standard deviation of distribution.

Table 1 shows the effect of the bend in the anterior descending branch of the left coronary artery. Before reaching the bend, sclerosis was measured as 47·5 *I/E* units; for the bend it was 59·8, 54·9 *I/E* units; and for the region following the bend, 48·4, 42·7, 32·9 *I/E* units. The higher value at the bend in the artery shows that this region is more susceptible to deposition.



(1)

(2)

Fig. 1. Early phase of experimental atherosclerosis of rabbit aorta. Note the plaques were beginning to form at each branching point. Flow of blood from left to right, as indicated by arrow. (× 3)

Fig. 2. Advanced phase of experimental atherosclerosis of rabbit aorta. Heavy plaques were formed at each branching point. Note the typical shape of the plaques in advanced sclerosis. Flow of blood from left to right, as indicated by arrow. (× 3)

The plaques along any artery are not randomly distributed as has been reported in the literature. They seem to be located at certain particular sites such as bifurcations. Fig. 1 shows a typical early phase of atherosclerosis which was formed at the branching point of an aorta from a rabbit given cholesterol in its feed. Fig. 2 shows another aorta with a comparatively advanced stage of atherosclerosis. Plaques are also particularly numerous at the branching point of an artery. In both photographs it is evident that plaques are formed at

precisely the same location with respect to flow. Theoretical and a full treatment of this particular feature will be published elsewhere.

Wei Young

Donner Laboratory of Medical Physics,
Department of Physics,
and
Lawrence Radiation Laboratory,
University of California,
Berkeley,
California.

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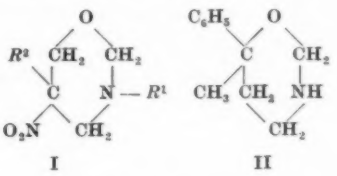
Anti-neoplastic Activity of Tetrahydro-1,3-oxazine Derivatives

It has been demonstrated that benz-1,3-oxazine derivatives inhibit the growth of Crocker sarcoma in mice[1]. This was partly confirmed by F. Bergel, at the Chester Beatty Research Institute, London (private communication).

Further confirmation of the anti-neoplastic activity of benz-1,3-oxazine derivatives was obtained by the Miyamura test, *in vitro*[2]. Similar results were obtained by Leonardi[3] when he repeated our experiments *in vitro*.

All these results seemed to be sufficiently promising to justify searching for anti-neoplastic substances among the derivatives of 1,3-oxazine. Simpler compounds, derivatives of 5-nitrotetrahydro-1,3-oxazines (I) and 6-methyl-6-phenyltetrahydro-1,3-oxazine (II) proved to be fairly efficient *in vitro*[2].



I II

$R^1 = CH_3$, $R^2 = CH_2$, *T* 398 (ref. 4) *T* 446 (ref. 7)
$R^1 = CH_3$, $R^2 = CH_2OH$, *T* 401 (ref. 4)
$R^1 = CH_3$, $R^2 = C_5H_{11}$, *T* 413 (ref. 5)
$R^1 = C_6H_{11}$, $R^2 = C_2H_7$, *T* 364 (ref. 6)

Their activity against the solid form of Ehrlich ascites carcinoma is shown in Table 1 and solid form of amytal ascites sarcoma in mice in Table 2.

It is believed that the active CH_2 group in position 2 is responsible for the anti-neoplastic activity of 1,3-oxazine derivatives.

Further experiments will be carried out with a number of the compounds (I) to elucidate the influence

Table 1. Inhibition of Ehrlich Ascites Carcinoma

Code No. of substance	Mouse strain	No. of mice in group	Daily dose of substance (mgm./mouse)	Average tumour weight (gm.)	$\sigma^* \pm g$	$m^\dagger \pm g$	Tumour inhibition (per cent)‡
T 364	*A*	12	0	2·4	0·79	0·23	
		10	12	1·3	0·52	0·16	45·8
	R III	12	0	2·7	0·79	0·23	
		10	12	1·3	0·94	0·29	51·8
T 398	*A*	12	0	2·4	0·79	0·23	
		10	12	0·8	0·56	0·18	66·7
	R III	12	0	2·6	0·79	0·23	
		10	12	0·8	0·51	0·16	69·2
T 413	*A*	12	0	1·8	0·50	0·14	
		10	12	0·9	0·57	0·18	50·0
	R III	12	0	1·5	0·57	0·16	
		10	12	0·5	0·22	0·07	66·7
T 446	*R* III	12	0	1·1	0·47	0·13	
		10	12	0·6	0·32	0·10	45·4

(See footnotes to Table 2).

Table 2. Inhibition of Amytal Ascites Sarcoma

Code No. of substance	Mouse strain	No. of mice in group	Daily dose of substance (mgm./mouse)	Average tumour weight (gm.)	$\sigma^* \pm g$	$m^\dagger \pm g$	Tumour inhibition (per cent)‡
T 364	*A*	12	0	2·3	0·45	0·13	
		10	12	1·0	0·39	0·12	56·5
	R III	12	0	1·8	0·71	0·20	
		10	12	0·8	0·41	0·13	55·5
T 398	*R* III	12	0	1·7	0·77	0·22	
		10	12	0·7	0·26	0·08	58·8
T 401	*A*	12	0	1·5	0·50	0·14	
		10	12	0·8	0·35	0·11	46·7
T 413	*A*	12	0	1·5	0·50	0·14	
		10	12	0·6	0·17	0·05	60·0
	R III	12	0	1·8	0·94	0·27	
		10	12	0·7	0·22	0·07	61·0
T 446	*R* III	12	0	1·7	0·66	0·19	
		10	12	0·7	0·39	0·12	58·8

$^* \sigma = \sqrt{\frac{\Sigma(x - \bar{x})^2}{n - 1}}$ $\quad$ $^\dagger m = \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n(n - 1)}}$

‡ Calculated according to the formula: $\frac{P - M}{P} 100$ = percentage inhibition. *P*, average tumour weight in control group; *M*, average weight in treated group.

of various substituents on their anti-neoplastic activity.

T. Urbański
D. Gürne
S. Ślopek
H. Mordarska
M. Mordarski

Organic Chemical Laboratories,
and
Institute of Immunology and
Experimental Therapy,
Polish Academy of Sciences,
Wrocław.

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BIOLOGY

Fungitoxicity of Metal Ions

The relationship between the toxicity of metal cations and their physico-chemical properties has been considered in terms of electrode potential[1,2], insolubility of the metal sulphide[3], and electronegativity[4]. Danielli and Davies[4] suggested that the toxicity of metal ions is determined by their ability to form un-ionized complexes with ionogenic groupings at the cell surface, and they have taken the electronegativity values of the metals as a measure of the tightness of covalent binding to these groups. The application of this hypothesis to the toxicity of a number of metal salts to fungal conidia[5] has recently been questioned by Miller[6]. In his examination of my experimental results, Miller has arbitrarily disregarded the toxicities of metals less toxic than zinc by terming them "basically not toxic to fungus conidia" although they all possess measurable *ED*50 values. It was, of course, precisely to show that the same toxic mechanism may apply to a wide range of metal ions that these less toxic metals were examined.

Miller also criticizes the standard spore germination technique[7] as a measure of fungitoxicity. This technique determines the fungistatic ability (that is, inhibition of spore germination) of a fungicide as a function of the concentration of the external solution, and is closely related to the practical performance of fungicides. Moreover, the widespread use of this test for many years has established its value as a measure of the relative toxicity of protectant fungicides. Absolute toxicity values will only be obtained when the concentration of toxicant at cell receptor sites can be determined. For some years, McCallan and Miller[8] have advocated the ratio of the weight of fungicide accumulated by the fungal spores at the *ED*50 to the weight of spores as a measure of 'innate' toxicity. Although their ratio of μgm. toxicant/gm. spore weight is a most valuable measure of fungicidal activity and of the uptake of fungicides by spores[9], this weight ratio cannot be regarded as fundamentally related to absolute toxicity until much more information on the mode of action of fungicides and their possible detoxication by the cell is available.

The value of the electronegativity hypothesis may best be shown by an examination of the results of some recent experiments in which the fungitoxicity of twenty-two metal cations to conidia of *Botrytis fabae* Sardiña has been determined. The median effective doses (*ED*50) of unbuffered solutions of nitrates of potassium, sodium, barium (*ED*50 extrapolated), strontium, lithium, calcium, magnesium, manganese, zinc, beryllium, thallium, yttrium, lead, chromium, nickel, cobalt, palladium, copper, and silver, of mercurous and mercuric chloride, and of osmium tetroxide were estimated as before[5]. *B. fabae* conidia gave 98–100 per cent germination after 18 hr. incubation at 25° in distilled water, so that a spore stimulant was not required. In Fig. 1 logarithms of the *ED*50 values of the metal ions are plotted against the electronegativities of the metals as calculated by Pauling[10] and Haïssinsky[11]. Haïssinsky calculated electronegativities by Pauling's method for the whole Periodic Table, including values for some of the different valence states of the transition elements. Although electronegativities are not in their nature quantities which can be defined precisely, modern work has largely corroborated Pauling's original values[12].

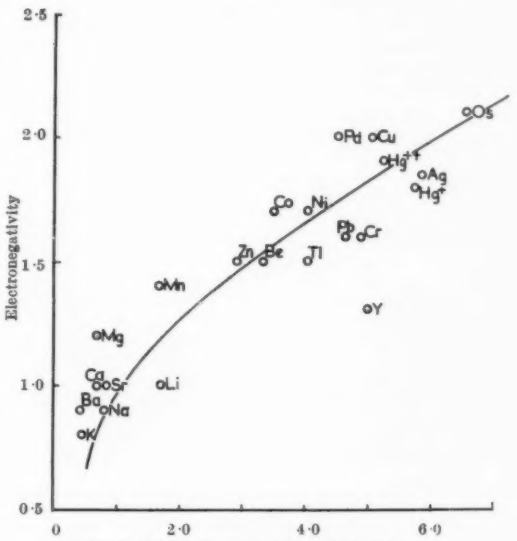

Fig. 1. Graph of toxicity of metal cations to *B. fabae* against electronegativity of the metal

Danielli and Davies[4] have shown that the values of Jones[3] for the toxicities of various cations to the planarian, *Polycelis nigra*, fit an exponential relationship of the form :

$$\log [M^{q+}] = \alpha - \gamma \exp(-0{\cdot}25x^2)$$

where $[M^{q+}]$ is the concentration of the metal cation of valency q in the bulk phase which gives the standard toxicity, x is the difference between the electronegativity of the metal and that of oxygen, and α and γ are constants. From the results in Fig. 1, $\log [M^{q+}]$ was plotted against $\exp(-0{\cdot}25x^2)$ and the regression line calculated, giving $\alpha = 1{\cdot}72$ (S.E., 0·59) and $\gamma = 13{\cdot}98$ (S.E., 1·48). These values of α and γ were used to plot the curve in Fig. 1 which, in spite of the anomalous position of yttrium, may be considered to

give a good fit to the experimental results. In view of the very different organism tested and the different electronegativities used, the values of α and γ are in reasonable agreement with those given by Danielli and Davies, namely, 0·95 and 9·28.

These results certainly suggest that there is a common, non-specific, toxic action for most metal cations which can be related to their strength of covalent binding to the surface ionogenic groups (for example, imidazole, carboxyl, phosphate, or sulphydryl) present outside the cell protoplast. It must be pointed out that there is a great deal of evidence from work with bacteria, yeast and fungi to show that the initial uptake of cations is a surface phenomenon[13]. The extent of metal accumulation by fungal spores is too great to assume a classical unimolecular adsorption layer[14], but rather an adsorption in depth on the surface matrix (ref. 13*a*). Danielli and Davies point out that the formation of an un-ionized complex between metal ion and surface ionic groups may alter membrane permeability; and Miller's results, showing increased uptake by fungal spores after treatment with silver ions or a surface-active agent, can be attributed to reaction between the metal ions and lysed cellular components. This reaction, however, will be secondary to the formation of un-ionized complexes, which is considered to be primarily responsible for the fungistatic action of metal cations.

The experimental work discussed here will be reported in detail elsewhere.

E. Somers

Long Ashton Research Station,
University of Bristol. March 14.

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A Method for the Detection of Inhibition of Auxin Transport

Experiments in which auxin has been supplied to either the morphologically apical or basal ends of coleoptile sections have been carried out by Åberg and Khalil[1] and by Housley *et al.*[2]. Both investigations demonstrated that more growth occurred when the apex-to-base auxin transport system could operate, that is, when the apical ends were in contact with the auxin. The present communication confirms this result and shows how the method can be used to investigate auxin transport and its inhibition.

Ten 1·0-cm. coleoptile sections cut from standard dark-grown wheat seedlings are supported upright inside a 1 in. × 1 in. vial containing 1·0 ml. of sucrose-buffer solution[3] with or without auxin, and in the presence and absence of a second substance such as 2,3,5-tri-iodobenzoic acid. The sections are placed so that the apical end (*A*-position), or the basal end (*B*-position), of each is just below the surface of the liquid. In addition, in certain experiments, sections are floated on the solutions (*F*-position). No special precautions are taken to guard against surface creep of solution along the sections, or against capillary rise between the coleoptile and the included leaf. Growth increments are measured after 18 hr. in darkness at 25° C. at high humidity.

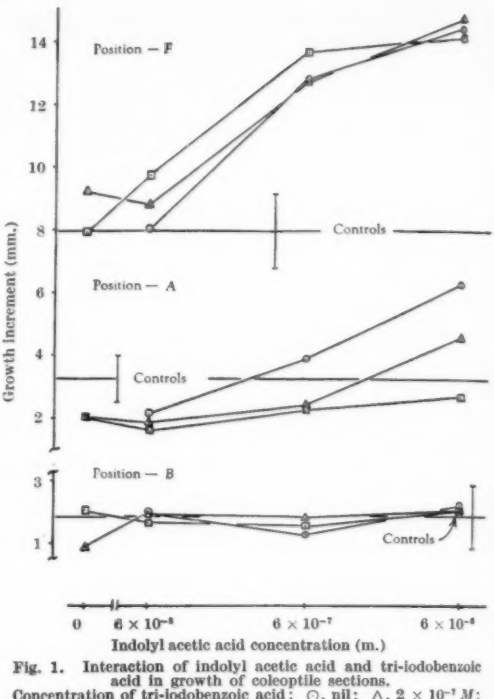

Fig. 1. Interaction of indolyl acetic acid and tri-iodobenzoic acid in growth of coleoptile sections. Concentration of tri-iodobenzoic acid: ○, nil; △, 2 × 10⁻⁷ *M*; □, 2 × 10⁻⁶ *M*

Sections in the *A*-position consistently show a greater growth response to indolyl acetic acid than sections in the *B*-position. This differential response is still apparent when: (*a*) the portion of leaf is removed, (*b*) the sucrose buffer is omitted from the medium (in which case all responses are lower), (*c*) each of the sections is cut into two 5-mm. portions, and both of these tested in both positions, and (*d*) oat coleoptile sections are used.

Fig. 1 shows results of an experiment on the interaction of indolylacetic acid and 2,3,5-tri-iodobenzoic acid. Points on the graphs represent growth increments found for sections in the *F*, *A* and *B* positions in different combinations of the two substances. Fiducial limits of controls, calculated from 5 sets of 10 sections, are shown.

In the *B*-position, externally applied indolyl acetic acid gives no growth response at the concentrations used. There is no significant difference between controls in the *A* and *B* positions; control growth might therefore be ascribed to the activity of endogenous auxin. In the *A*-position, growth responses to exogenous auxin do occur, although they are less than in floating sections. It is suggested that

responses in the *A*-position represent an assay of transported indolyl acetic acid. Where tri-iodobenzoic acid is present, they are reduced or absent. Since these concentrations of tri-iodobenzoic acid do not interfere with growth induced by indolyl acetic acid in the *F*-position, it can be argued that the reduction they cause in *A*-position responses is due to an inhibition of auxin transport, rather than to effects of tri-iodobenzoic acid on auxin uptake and assay. Such effects have been tested and corrected for by other workers[4,5], who have likewise found that tri-iodobenzoic acid inhibits auxin transport. The same argument applies to destruction of auxin induced by tri-iodobenzoic acid, which has been shown to occur[5,6] at concentrations higher than those found here to inhibit transport.

Experiments of this type may prove suitable for the testing of tissue extracts for substances that might regulate some aspects of plant growth through their effects on the transport of auxin. There is direct evidence for the existence of transport-modifying substances[7], and their activity can be postulated as a working hypothesis to account for a variety of correlation phenomena. Substances already available in a pure form can also be examined. For example, the method has shown that colchicine, in concentrations (0·001 and 0·01 per cent) that are within the range causing apolar cell enlargement in root 'c-tumours', does not behave in the same way as tri-iodobenzoic acid.

B. E. S. Gunning

Department of Botany,
Queen's University, Belfast.

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Maternal Inheritance and Interspecific Hybrids of the Liverwort *Sphaerocarpos*

Sex determination in *Sphaerocarpos*, a genus of dioecious liverworts, is genetically controlled[1]. When the spores of a tetrad derived from a single spore mother cell are germinated, two of the sporelings develop into male and two into female gametophytes. Cytological studies have shown that there are eight chromosomes in the complement of each sex. Of these, seven of the eight chromosomes in the female are similar to seven in the male. The eighth chromosome of the female, the *X* chromosome, is larger than the seven autosomes; the eighth chromosome of the male, the *Y* chromosome, is smaller than the autosomes[2–4].

Of the seven species in the genus, there are no readily apparent morphological differences separating the male plants. Moreover, only one species is easily identified on the basis of the vegetative characteristics of its female plant[4]. Although some vegetative differences are said to exist between the female plants of the other six species, apparently these differences are difficult to ascertain in field populations. Therefore, these species are usually separated on the basis of spore characteristics, that is, the pattern of spore markings and whether or not the four spores remain adherent in a tetrad after meiosis. Recently, in the work reported here, it was found that these spore characteristics are isolated from the effects of hybridization. That is, hybrid spores are not intermediate between the two parents, but closely resemble the maternal species.

Three species of *Sphaerocarpos* were used during the present work: *S. donnellii* Austin (from Florida), *S. drewii* Wigglesworth (from California) and *S. cristatus* Howe (from California). The spores of *S. drewii* and those of the particular race of *S. donnellii* used in this work are adherent in a tetrad; those of *S. cristatus* are separate. Sexual reproduction between these species is normally precluded by geographical isolation. Clonal cultures of each sex, derived from single-spore isolates, were grown separately. Two separate matings involving reciprocal crosses between the three species were attempted. Simultaneous intraspecific matings served as controls and also indicated the number of archegonia with eggs receptive to fertilization at the time of each mating. Between 60 and 105 archegonia contained receptive eggs in each control culture.

Of concern here are only those crosses which resulted in the development of mature sporophytes and thus mature spores. Mature hybrid sporophytes resulted from the following matings: *S. cristatus* ♀ × *S. donnellii* ♂; *S. drewii* ♀ × *S. cristatus* ♂; and *S. donnellii* ♀ × *S. cristatus* ♂. Only in matings of *S. cristatus* ♀ × *S. donnellii* ♂ did the number of sporophytes produced compare to that for the control, *S. cristatus* selfed. Considerably fewer sporophytes resulted from the mating between *S. drewii* ♀ × *S. cristatus* ♂ than from the control, *S. drewii* selfed. The second mating between *S. donnellii* ♀ × *S. cristatus* ♂ resulted in a single sporophyte which produced morphologically normal spore tetrads.

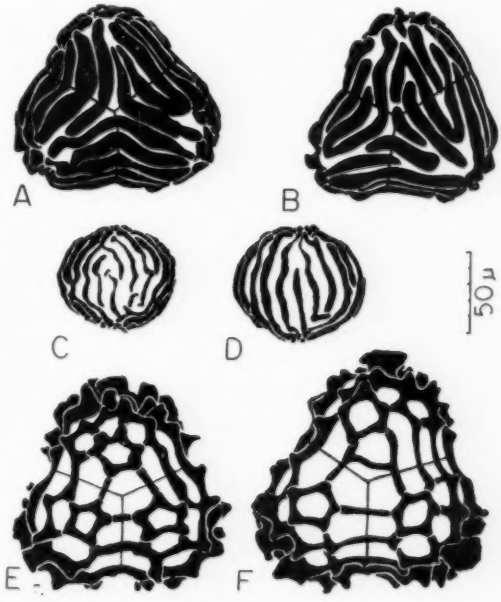

Fig. 1. Spores and spore tetrads of *Sphaerocarpos*. Spores of the maternal species are on the left; those of the hybrids on the right. *A*, *S. drewii*; *B*, *S. drewii* ♀ × *S. cristatus* ♂; *C*, *S. cristatus*; *D*, *S. cristatus* ♀ × *S. donnellii* ♂; *E*, *S. donnellii*; *F*, *S. donnellii* ♀ × *S. cristatus* ♂

Spore characteristics of the parent species and the hybrids are depicted in Fig. 1. All hybrid spores within a single sporophyte and in different sporophytes of the same mating are essentially similar to those shown. It is evident that the hybrid spores have both the general and the definitive characters of the maternal species. Similar results were reported by Allen[5] in the cross between *S. texanus* Austin ♀ × *S. donnellii* ♂. In addition, Allen[6] found that within the species *S. donnellii* the character of adherent versus separate spores followed the line of descent of the *X* chromosome. Although the possibility of cytoplasmic inheritance through the egg cannot be entirely disregarded (see Wettstein[7] for cytoplasmic inheritance in mosses), the most plausible explanation for this pattern of inheritance is that the spore morphology is determined by the genetic composition of the *X* chromosome.

Naturally occurring hybrids have not been found in liverworts[8]. In *Sphaerocarpos* it is clear that naturally occurring hybrids would not be recognized as long as spore characteristics are equated with specific identity. These hybrids would routinely be referred to the maternal species. This means that hybridization will have no taxonomic meaning until species identification includes the use of additional, non-spore characters. It would be of interest to learn how many of the reported differences between the female plants of the species of *Sphaerocarpos* are sex-linked, how many are determined by autosomes and how many are determined by the environment. A critical re-examination of the genus is indicated, utilizing cultures grown under uniform laboratory conditions.

William T. Doyle

Northwestern University,
Evanston, Illinois.

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Significance of the Type of Host Plant Crop in Successful Biological Control of Insect Pests

Recent publications[1–3] on the status of biological control of insect pests have revealed widely divergent opinions on the potentialities of this method of pest suppression. The fact that in the past a high percentage of successful results has occurred in tropical islands led Taylor[1] to conclude that "there is little future for it in continental areas", and to re-emphasize an earlier thesis of Imms[4], to the effect that only such insular conditions are basically suited for this technique. This opinion was rejected by Simmonds[2], whose point of view is supported by Clausen[3,5] on the basis of reviews of work done in North America during the past twenty-five years.

In these and other publications[6–8] bearing on the prerequisites of successful biological control practice, no reference is made to an environmental factor which appears to have been of significance in island and continental experiments. All cases where introductions of natural enemies are reported by competent authorities[9,10] to have given full commercial control of the pests, and the majority of those where control has been partial pertain to perennial crops. More specifically these plants have been sugar-cane, alfalfa, coconut, citrus, coffee and sundry deciduous fruit, forest or shade trees. The following examples may be cited: the sugar-cane weevil *Rhabdoscelus obscurus* (Boisduval) and the leaf-hopper *Perkinsiella saccharicida* Kirkaldy by *Microceromasia sphenophori* (Villeneuve) and *Cyrtorhinus mundulus* (Breddin) in Hawaii; the coconut moth *Levuana iridescens* Bethune-Baker, the scale *Aspidiotus destructor* Signoret, and the leaf-miner *Promecotheca reichei* Baly by *Ptychomyia remota* Aldrich, *Cryptognatha nodiceps* Marshall, and *Pleurotropis parvulus* Ferriere, respectively, in Fiji; the citrus pests *Icerya purchasi* Maskell, and *Pseudococcus gahani* Green by *Rodolia cardinalis* (Mulsant), *Coccophagus gurneyi* Compere and *Tetracnemus pretiosus* Timberlake in California; the citrus blackfly *Aleurocanthus woglumi* Ashby by *Eretmocerus serius* Silvestri in the West Indies and by *Amitus hesperidum* Silvestri and *Prospaltella* spp. in Mexico; the citrus whitefly *Aleurocanthus spiniferus* (Quaintance) by *Prospaltella smithi* Silvestri in Japan; the coffee mealybug *Planococcus kenyae* Le Pelley by *Anagyrus kivuensis* Compere in Kenya; the woolly apple aphid *Eriosoma lanigerum* (Hausmann) by *Aphelinus mali* (Haldeman) and the blue gum scale *Eriococcus coriaceus* Maskell by *Rhizobius ventralis* Erichson in New Zealand; the larch sawfly *Pristophora erichsonii* (Hartig) by *Mesoleius tenthredinis* Morley in Canada; and the eucalyptus snout weevil *Gonipterus scutellatus* Gyllenhall, by *Anaphoidea nitens* Girault in New Zealand, South Africa, Madagascar and Mauritius.

Consideration of these and the many other attempts to utilize biological agents leads to the conclusion that these agents have been particularly effective where the host plants are trees or other perennials maintaining their geographical locations over periods of many years. The case of sugar-cane is somewhat exceptional in that there is frequent cutting to ground-level. But since maturity is reached 10–20 months after planting, crops overlap, and ratooning is common at least under moist tropical conditions, the plants provide an approach to the more stable and permanent conditions in the tree crops. Indeed, some authors[11] have actually suggested that tall canes maintain an environment more in common with a forest than a savannah since many sugar-cane pests appear to have been originally forest inhabitants. Where sugar-cane is grown in marginal climatic areas such as Louisiana and Argentina, the length of the frost-free season is critical and the crop is cut annually. Under these conditions control of pests by natural enemies has not matched that obtained in the tropics.

The special suitability of tree crops as environments for the action of biological agents is seen also in the results of long-term field studies on biological balance in California avocado orchards by Fleschner[12,13]. It is seldom necessary to apply chemical pest control treatment in these orchards; but Fleschner showed by numerous experiments that when entomophagous insects were excluded from trees in any area phytophagous mite and insect populations increased to damaging levels. A further illustration of the great significance of stable environments in control programmes involving living organisms is apparent on examination of experiments in the biological control of weeds by insects. Outstanding successes to date have been against perennial weeds of undisturbed lands[14].

In contrast to pests of tree or other perennial crops, field and garden insects attacking plants such

as cereals, maize, crucifers, legumes, etc., have proved to be far more refractory to adequate control by biological agents. So much so that in published lists of pests brought under full commercial control by biological means the omission of examples from these crops is striking. Of course, partial control of a quite valuable nature has been obtained in some field crops, but here again the importance of the perennial plant is evident. The introduction of *Pteromalus puparum* (Linnaeus) and *Angitia* spp. against the cruciferous pests *Pieris rapae* (Linnæus) and *Plutella maculipennis* (Curtis) in New Zealand; *Ascogaster quadridentata* Wesmael and *Glypta haesitator* Gravenhorst against pea moth *Laspeyresia nigricana* (Stephens) in British Columbia; *Euplectrus plathypenae* Howard and *Archytas cirphis* Curran against pasture cutworms *Pseudaletia unipuncta* (Haworth), etc., in Hawaii; and *Praon palitans* Muesebeck, *Trioxys utilis* Muesebeck and *Aphelinus semiflavus* Howard against spotted alfalfa aphid *Therioaphis maculata* (Buckton) in California[15], are cases in point. Biological control programmes against pests of field and garden crops have often been pursued with a vigour equalling efforts applied to programmes against tree crop pests, as may be seen by the examples cited in Clausen's review of biological control work in the continental United States[5]. The sharp differences in the end-results of these numerous empirically conducted projects during the past seventy years thus suggest a real limitation in the biological control method. Until research shows how such environments may be made more favourable for the action of biological agents, major attention might profitably be directed to pests of perennial crops, where full commercial control is sometimes attainable.

The reasons for these different results are not immediately apparent, although the differences are understandable when the projects are considered from behaviouristic and ecological points of view. The effective environment of an organism has been defined as that containing everything of direct importance to the particular organism. It is probable that tree and other perennial crops provide better approximations to this environment than do field crops because of their fortunate combination of extension, continuity and fixity. These relatively stable and undisturbed conditions year after year must inevitably facilitate sensory reactions of biological agents such as adult entomophagous insects. The overall capacity of an environment to meet the specialized requirements and limitations of these adults may well be a critical, even decisive, factor in the ultimate success of a biological control programme. Really effective natural enemies have been shown to disperse rapidly and have good host-searching capacities. These faculties involve many sense organs, and prevailing opinion is that the adult is attracted first to the type of environment or host area, and then to the host insect[16]. The relative failure of biological control agents against field and garden crop pests suggests the possibility that the organic sensory equipment of the adult entomophagous insect lacks the capacity to cope with the constant disruptions of these plant environments by the cropping and other sequences of present-day agricultural systems. Continuous cultivation under one crop has long been known to favour accumulation of pests, whereas a shifting cultivation or rotation system helps check them. It is to be expected that these same practices create corresponding difficulties in the maintenance or augmentation of the natural enemy populations of the pests. Successful attempts to alter these environments in favour of the biological agents might well follow from an increased knowledge of the factors controlling the orientation and movement patterns of the entomophagous insects and other natural enemies.

D. C. Lloyd
Commonwealth Institute of Biological Control,
P.O. Box 575, California Station,
Fontana, California.

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Diplodiscus Species Parasitic in the Snail *Paludina vivipara* Lm.

While engaged in researches on larval trematodes in The Netherlands, I collected specimens of the snail *Paludina vivipara* Lm. from a dried-up waterway in the vicinity of Houten (Province of Utrecht). In the course of routine examination, no cercariæ having been given off by the snails under laboratory conditions, they were dissected and large numbers of rediæ found which proved, by their structure and measurements, to be the larval stage of an amphistome. This is in itself unusual as *P. vivipara* is not recorded as acting as intermediate host for this group of trematodes.

Further dissection and investigation revealed in two of the snails the presence of active trematodes, associated with the tissues of the gut-wall. On investigation, these proved to be adult amphistomes of the genus *Diplodiscus* Diesing 1836, known previously only from vertebrates (Amphibia). Of the members of the genus, the new amphistome most closely resembles *Diplodiscus subclavatus* (Pallas).

The accompanying photograph (Fig. 1) shows the structure and state of development of the amphistome, and its resemblance to *Diplodiscus* sp. is clear. A feature of interest is that only one testis is present, which indicates that the trematodes are in fact adults, young specimens having a double testis which later fuses. The dark paired structure (black in the photograph) is the excretory system.

The measurements of the trematode shown are as follows (in microns):

Body-length	2,320
Breadth, at *A*	710
Oral sucker	290 × 260
Acetabulum	860 × 610
Testis diameter	300

At present, there appear to be two explanations for the occurrence. One one hand, it is possible that the larval stages of the amphistome have taken place in another mollusc, for example *Planorbis* sp., and that the subsequent eating by *Paludina vivipara* of encysted metacercariæ (perhaps by eating a dead

Fig. 1. Measurements given in text

frog or another mollusc) has given the opportunity for full development within a molluscan host, in place of one of the expected vertebrate host (that is, one of the Amphibia).

On the other hand, the presence of amphistome rediæ raises the possibility that the whole of the life-cycle may occur, by means of a type of 'short-circuiting', within the (former) intermediate host, which would account for the lack of free-swimming cercariæ. The cercaria of *Diplodiscus subclavatus*, in contrast, is well known under the name *Cercaria diplocotylea* Fil. Although this has a period of development external to the redia (that is, in the tissues of the snail *Planorbis umbilicatus* L.), it later leaves the intermediate host, and encysts externally.

It is of interest to speculate on the definitive host for this species of *Diplodiscus*, and whether it is a parasite of molluscs, in contrast to all other members of the group, or a parasite of vertebrates that, in exceptional circumstances, has adapted itself to a molluscan host. Whatever the answer to this problem may prove to be, it is the first known case of an amphistome parasitizing an invertebrate.

For the opportunity and facilities to carry out further work on the life-cycle and identification of this trematode, the results of which it is hoped to publish at a later date, I am indebted to the Institute mentioned below and in particular to its director, Prof. D. Swierstra.

M. R. Honer
Institute for Parasitology and Parasitic Diseases,
State University, Biltstraat 172,
Utrecht.

Effect of Pyruvate upon Nucleated and Enucleated Fragments of *Amoeba proteus*

The detection of metabolic differences between nucleated and enucleated fragments of *Amoeba proteus* suffers from the fact that the amounts involved are often minute[1]. In a search for the effect of enucleation upon cellular metabolism, the sensitivity of nucleated and enucleated fragments, respectively, towards selected metabolites may serve as a guide.

The effect of pyruvate on nucleated and enucleated fragments of *Amoeba proteus* was tested as one example. Since enucleated cells are unable to take up food particles, both the controls and the fragments were kept in non-nutrient salt solution without the addition of food particles throughout the experiment. The *Amoebae* were cut into nucleated and enucleated halves with a glass needle. Nucleated fragments as well as whole cells tolerated concentrations of sodium pyruvate up to $2 \cdot 5 \times 10^{-3}$ mM/ml. for 24 hr. without appreciable changes either in their mode of movement, or in the morphology of the pseudopodia. However, about 90 per cent of the enucleated fragments were cytolysed within the first 10 hr. of exposure to the same concentration. This difference is the more remarkable since both nucleated and enucleated fragments survive starvation for more than a week.

Since synthesis of protein and ribonucleic acid is dependent on the nucleus, the higher toxicity of the pyruvate for the enucleated fragments might reflect an intracellular increase in lactic acid, caused by a change in the reduced diphosphopyridine nucleotide/diphosphopyridine nucleotide ratio as a result of an insufficient consumption of chemically bound energy[2,3]. It is interesting that a similarly increased sensitivity of enucleated fragments towards 'natural' precursors of proteins and nucleic acids has been found in *Acetabularia*[4].

Edmund Guttes
Sophie Guttes

Department of Biology,
Brown University,
Providence,
Rhode Island.

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BACTERIOLOGY

Egg-Yolk Reactions of *Pseudomonas* Species

Various bacteria are known to produce opalescence in egg-yolk emulsions due to lecithinase or lipase activity. Among these are the lecithinase-producing clostridia and aerobic spore-forming bacilli, and the lipolytic clostridia and staphylococci. Little attention seems to have been paid to the effects produced by Gram-negative organisms growing on egg-yolk media. Felsenfeld[1] showed that the opalescence produced by four strains of *Vibrio comma* and one strain of *Vibrio el tor* was due to lecithinase activity, and Crook[2] made a passing reference to one strain of *Pseudomonas pyocyanea* (*Ps. aeruginosa*) which produced an opalescence on egg-yolk agar. Willis[3] noted the opalescence changes produced by four strains of *Ps. pyocyanea*.

This communication presents briefly the results obtained in a study of the cultural reactions of *Pseudomonas* species on egg-yolk agar and other lecithin and fat-containing media. Thirty strains were examined, of which 15 were strains of *Ps. pyocyanea* isolated from human infections; the remainder consisted of a number of species other than *Ps. pyocyanea*.

All organisms were examined for lipolytic activity by growing them on fat-containing media (tributyrin agar ('Oxoid'), cream agar, monostearate agar and

tristearate agar), and for lecithinase activity on egg yolk agar and lecithin agar. Fat-free milk agar was used to determine proteolytic activity, and nutrient agar without other additions was used as a control medium. Some of these media have been described by Willis[3]. In addition, the strains were cultured on phenolphthalein phosphate agar to determine their phosphatase activity, and a note was made of their temperature requirements, pigment production and pathogenicity for mice.

All the strains of *Ps. pyocyanea* produced marked clearing on tributyrin agar in 48 hr., and obvious lipolysis on cream agar, monostearate agar and tristearate agar as indicated by positive results in the copper soap test for lipase[3,4]. Pancreatic lipase (Light and Co.) and four known lipolytic coagulase-positive strains of *Staphylococcus aureus* produced identical results. All strains were proteolytic on milk agar, and, apart from pigment production which varied from strain to strain, they produced no change on nutrient agar. Most strains were phosphatase-positive and were pathogenic for mice.

On the lecithin-containing media—egg-yolk agar and lecithin agar—all strains produced an opalescence and commonly a pearly layer also. On egg-yolk agar the opalescence was almost always confined to the limits of growth; but on lecithin agar it commonly extended away from the edge of the growing colony for some distance into the medium. After treatment with saturated copper sulphate solution the central parts of the opalescence in all cases stained greenish blue, whereas the peripheral parts remained unstained. This was most obvious in the cultures on egg-yolk agar. Pancreatic lipase and the four strains of *Staph. aureus* produced no change on lecithin agar, but produced an intense opalescence on egg-yolk agar and a strongly positive result in the copper soap test on this medium. Pancreatic lipase and the staphylococci also gave positive results in the copper soap test on lecithin agar which had been treated with *Cl. welchii* α-toxin (lecithinase *C*).

Pseudomonas species other than *Ps. pyocyanea* acted variously on the different media. In general, those which grew at 37° C. gave similar results to those outlined for *Ps. pyocyanea*, while those which grew at 25° C. but not at 37° C. were comparatively inactive.

There is no doubt that many of the strains of *Pseudomonas* species examined are lipolytic, and this activity would account for the opalescence produced by them on egg-yolk agar. However, the fact that these organisms produce an opalescence on lecithin agar which stains with copper sulphate solution, whereas lipolytic staphylococci and pancreatic lipase produce no change in the medium, suggests that they produce also a lecithinase *C*. Lipolysis of the diglyceride liberated from the lecithin molecule by attack by lecithinase *C*, as occurs in the case of *Clostridium oedematiens* type *A*[3], provides the simplest explanation of the results obtained.

Further investigations along these lines are at present being carried out and a detailed report will be published elsewhere.

A. T. Willis
G. Gowland

Department of Bacteriology,
School of Medicine,
Leeds. March 18.

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Two Types of Catalase-like Activity in Lactic Acid Bacteria

When heterofermentative lactic acid bacteria were being tested for hydrogen peroxide production, using adaptations of Kneteman's[1] pyrolusite medium and the blood benzidine medium of Kraus, Nickerson, Perry and Walker[2], it was noted that one group showed a positive reaction on both media, and another group produced peroxide on the pyrolusite medium only. Later, it was found that the latter group, but not the former, possessed a strong catalase-like activity when hydrogen peroxide (10 vol.) was added to the cultures grown on blood media; no such reaction was observed when the cultures were grown on blood-free media. Further experiments were carried out with a basal medium (0·5 per cent Evans peptone, 0·5 per cent 'Lab-Lemco', 5·0 per cent yeast autolysate, 0·05 per cent manganese sulphate, 0·05 per cent 'Tween 80', 0·5 per cent potassium citrate, 1·5 per cent agar, and 0·5 per cent glucose, unless otherwise stated, *p*H 6·5 and autoclaved at 15 lb. for 20 min.) to which blood, lysed by the addition of an equal amount of sterile tap water, was added (5 per cent final concentration). This medium, boiled for 15 min. to destroy the blood catalase before pouring into plates, was inoculated by streaking the surface and incubated aerobically at 30° C. for two days. The evolution of bubbles from bacterial cells on the addition of hydrogen peroxide was accepted as an indication of catalase-like activity. Again the cultures were strongly catalase-positive; the medium itself did not decompose peroxide. Catalase-like activity could also be induced by flooding with hæmin (50 μgm. per ml. hæmatin–hydrochloric acid) catalase-negative cultures, grown on the basal medium, and testing after incubation for a further hour.

A selection of lactic acid bacteria was then examined for catalase-like activity on heated-blood agar and on the basal medium, inoculated and incubated as before. Glucose was replaced by arabinose, fructose, or xylose for certain strains which grew feebly with glucose as the energy source. The identity of the heterofermentative lactobacilli, the *casei-plantarum* group of lactobacilli, the leuconostocs, and the pediococci, was determined so far as possible by physiological and morphological tests. The remaining organisms are listed under the names they had when received from the sources indicated in Table 1. The results (Table 1) show that the property of forming a catalase-like system, in the presence of heated blood, is possessed by a number of strains. A few strains of pediococci, however, were weakly catalase-positive on both heated-blood and blood-free media and are not, therefore, recorded in the table as having catalase-like activity only on heated-blood medium. All other strains of lactic acid bacteria examined were either catalase-negative on the heated-blood and on the basal media or developed a catalase-like activity on the heated-blood medium only. A few strains of *Leuconostoc* and *Lactobacillus plantarum*, National Collection of Dairy Organisms (N.C.D.O. 704), were inhibited in the presence of blood. In these cases cultures on the basal medium were flooded with hæmin and tested as described previously. All were catalase-negative.

These results led to further work on the nature of the hæm-requiring catalase-like system. A washed cell suspension of *L. plantarum*, National Collection of Industrial Bacteria (N.C.I.B. 5914), which is

Table 1. Catalase-like Activity on Heated-Blood Agar

Organism and No. of cultures	Source	No. catalase-positive
Genus *Lactobacillus*		
L. acidophilus (2)	N.C.I.B.	0
L. bulgaricus (1)	,,	0
L. lactis (1)	,,	0
L. thermophilus (1)	,,	0
L. leichmanni (3)	,,	0
L. delbrückii (4)	,,	0
L. helveticus (1)	N.C.D.O.	0
L. salivarius (2)	Dr. M. Rogosa and Dr. M. E. Sharpe	0
L. casei (8)	N.C.I.B., N.C.D.O., Dr. R. M. Keddie and own isolates	1 (N.C.D.O. 161)
L. plantarum (22)	N.C.I.B., N.C.D.O., Dr. R. M. Keddie and own isolates	20 (N.C.I.B. 6105, N.C.D.O. 704, negative)
L. buchneri (22)	N.C.I.B. and own isolates	0
L. brevis (30)	N.C.I.B., Dr. M. E. Sharpe and own isolates	28 (N.C.I.B. 4036, 4617, negative)
L. fermenti (4)	N.C.I.B. and N.C.D.O.	0
L. cellobiosus (2)	Dr. M. Rogosa	2
L. viridescens (2)	Dr. M. E. Sharpe	0
L. fructivorans (2)	N.C.I.B.	2
L. hilgardii (1)	,,	0
Unidentified (18)	Dr. T. Gibson and own isolates	0
Genus *Leuconostoc*		
L. mesenteroides		
Non-slime formers (16)	N.C.D.O., Dr. T. Gibson and own isolates	8 (own isolates)
Slime formers (60)	N.C.I.B., N.C.D.O. and own isolates	0
L. dextranicum (7)	N.C.I.B., N.C.D.O. and own isolates	0
L. citrovorum (4)	N.C.D.O.	0
Genus *Streptococcus*		
S. faecalis (12)	N.C.I.B., N.C.D.O., Dr. P. M. F. Shattock and own isolates	0
S. faecium (10)	N.C.D.O., Dr. P. M. F. Shattock and own isolates	0
S. liquefaciens (3)	N.C.D.O. and own isolates	0
S. zymogenes (2)	N.C.D.O.	0
S. durans (2)	,,	0
S. thermophilus (2)	,,	0
S. lactis (4)	,,	0
S. cremoris (4)	N.C.D.O. and N.C.I.B.	0
Genus *Pediococcus*		
Various strains (21)	N.C.I.B., stock cultures and own isolates	3 (N.C.I.B. 8018 and two stock cultures)

catalase-positive on heated-blood agar, was prepared from an aerobic culture on the basal medium; the cells then showed no catalase activity. Within 5 min. of the addition of hæmin solution (5 μgm. per ml. hæmatin–hydrochloric acid) they developed a strong catalase-like activity. Holding the cells alone in boiling water followed by the addition of hæmin, or boiling the cells 10 min. after the addition of hæmin, resulted in their failure to acquire a catalase-like activity. Cell suspensions of *L. casei* N.C.I.B. 4113, which is catalase-negative on the heated-blood medium, did not become catalase-positive in the presence of hæmin. These findings suggest that certain lactic acid bacteria possess a protein capable of coupling with a hæm compound to form a system which actively splits hydrogen peroxide. This system did not appear to be sensitive to acidity, since a representative selection of strains exhibited a strong catalase-like activity when they were grown aerobically on heated-blood media at an initial *p*H of 7·0, 5·4 and 4·5. Anaerobic cultural conditions, however, did lessen the peroxide-splitting activity of a few strains of heterofermentative lactic acid bacteria.

As mentioned earlier, some pediococci were weakly catalase-positive on several media. Therefore all the heterofermentative organisms, the *plantarum-casei* group of lactobacilli, the streptococci, and the pediococci, were examined for catalase activity on the basal medium containing 0·05 per cent glucose. Only six slime-forming strains of *Leuconostoc mesenteroides*, and ten of the pediococci showed catalase activity, the leuconostocs being less active than the pediococci. The catalase activity of all six leuconostocs and five of the pediococci decreased as the glucose concentration was increased. This decrease in catalase activity agrees with previous findings for pediococci[3] and *Lactobacillus plantarum*[4]. The presence of heated-blood or hæmin had no effect upon the activity, which suggests that two distinct catalase-like systems occur in the lactic acid bacteria. A study of the effect of initial *p*H and oxygen, using 0·05 per cent glucose basal medium, revealed that the catalase-like activity of the leuconostocs diminished with lowering of *p*H and was absent altogether in anaerobically grown cultures, except for one strain which exhibited a very weak activity after being allowed to stand for 1 hr. in contact with the atmosphere before the addition of peroxide. The catalase-like activity of the pediococci was just as apparent in cells grown aerobically or anaerobically; it was influenced by *p*H but not uniformly in all strains. The strains showing activity in 0·5 per cent glucose media were only slightly affected by a *p*H (initial) as low as 4·5. Other strains, catalase-negative in 0·5 per cent glucose media, were as sensitive as the leuconostocs to *p*H.

There appears, therefore, to be at least two recognizable forms of catalase-like activity in the series of lactic acid bacteria examined. The first is a vigorous hydrogen-peroxide destroying system, not sensitive to acid and requiring an external source of hæm. It is possessed by certain strains of homo- and heterofermentative lactobacilli, pediococci, and leuconostocs. The second is a much weaker, often acid-sensitive system, possessed by further strains of leuconostocs and pediococci. A catalase-like activity similar to the latter type has been recorded previously for the pediococci[3], strains of *L. plantarum*[4], and possibly, though no details were given[5], for non-slime and slime-forming strains of *Leuconostoc mesenteroides*, and additional strains of *L. plantarum*.

This, and further work, will be reported in more detail elsewhere. I wish to express my thanks to Dr. T. Gibson for his advice during this work, to Dr. R. M. Keddie for his criticism, and to those who kindly sent me cultures.

R. Whittenbury

Department of Bacteriology,
Edinburgh School of Agriculture,
West Mains Road,
Edinburgh 9.

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Production of Tuberculin Sensitivity

Delayed, or tuberculin-type, sensitivity occurs in the course of many bacterial infections. It is characterized by skin reactions appearing with 24–48 hr. after intracutaneous injections of bacteria or their soluble products. Like immunological reactions, delayed skin reactions are highly specific, but the products eliciting the reactions need not be antigenic *per se*. Circulating antibodies have not been demonstrated to play a part. Delayed sensitivity is not transferable passively by serum, but can be transferred with the cells of sensitized organisms.

Table 1. TUBERCULIN REACTIONS IN GUINEA PIGS SENSITIZED WITH PURIFIED PROTEIN DERIVATIVE OF TUBERCULIN ADSORBED TO EXUDATE CELLS

Guinea pig No.	0 hr.	4 hr.		24 hr.		Active cutaneous anaphylaxis†	Passive cutaneous anaphylaxis
	Skin thickness*	Skin thickness	Diameters of erythema (mm.)	Skin thickness	Diameters of erythema (mm.)		
1	30	36	9 × 10	42	13 × 14	—	—
2	24	29	10 × 11	31	10 × 12	—	—
3	27	34	7 × 7	36	16 × 16	—	—
4	30	32	6 × 7	35	8 × 10	—	—
5	32	44	10 × 12	44	9 × 11	—	—
6	30	35	8 × 9	42	11 × 12	—	—
7	27	29	5 × 5	30	9 × 11	—	—

* The thickness of a skin-fold was measured with callipers. The calliper readings which appear in the table are multiples of 0·1 mm.
† This test was performed on a different set of animals immunized in exactly the same way. — indicates that the test was negative.

Delayed sensitivity to tuberculin occurs regularly after infection with virulent or attenuated tubercle bacilli as well as after the injection of killed mycobacteria. It can also be produced by injecting native tuberculo-protein along with certain wax fractions of tubercle bacilli[1].

Delayed sensitivity seems to occur mainly when the antigenic materials have been phagocytosed (as is the case in bacterial infections) and there appears to exist a certain correlation between the intracellular state of micro-organisms and their ability to cause delayed sensitivity. An attempt was therefore made to sensitize animals with tuberculin by binding it firmly to phagocytes and using these treated cells to sensitize animals. For these experiments, a purified protein derivative of tuberculin, batch *RT* 23 (Statens Seruminstitut, Copenhagen, Denmark), was chosen. This preparation is widely used for tuberculin testing of humans.

A typical experiment is described. Normal guinea pigs were injected intraperitoneally with 10 ml. of a 0·0001 per cent solution of glycogen in saline. Four days later, the exudates consisting mainly of monocytes were collected from the peritoneal cavities. The cells were washed with heparinized Tyrode solution and resuspended in Tyrode. The average yield was about 1×10^8 cells per guinea pig. 10 ml. of pooled exudates from 4–6 animals were incubated with 10 mgm. purified protein derivative of tuberculin for 40 min. at 37° C. The cells were then washed five times in Tyrode to remove all non-adsorbed derivative. Studies with the purified protein derivative of tuberculin labelled with chromium-51 (ref. 2) showed that 10^8 cells adsorbed about 0·1 mgm. of the derivative. The washed, sensitized cells were suspended in Tyrode, and new guinea pigs, weighing about 350 gm., were injected into the footpads with approximately 10^8 cells in 1·0 ml. Tyrode.

Twelve days later the guinea pigs were tested intracutaneously with 250 T.U. purified protein derivative of tuberculin. Reactions were read at 4–5, 24 and 48 hr. Table 1 shows the results. The majority of the reactions had all the characteristics of tuberculin reactions. They were indistinguishable in their appearance from the reactions observed in control guinea pigs which had been sensitized at the same time with living *BCG*.

Two guinea pigs (Nos. 2 and 5) had reactions which seemed to be mixed Arthus and tuberculin reactions, appearing earlier but persisting as long as the pure delayed reactions. In another, similar experiment 31 days were permitted to elapse between sensitization and tuberculin testing. The guinea pigs, there too, showed typical delayed reactions.

Similar skin reactions were observed when guinea pigs were tuberculin tested 12 days after they had been injected with purified protein derivative of tuberculin (0·1 mgm. in 0·1 ml. saline) into the centre of a sterile abscess produced four days previously by an intramuscular injection of 0·1 ml. turpentine into the thigh. However, tuberculin sensitivity failed to occur when normal guinea pigs were injected with the derivative directly into the footpads, or into the skin or, intraperitoneally, into preformed four-day-old exudates. Injection of the derivative adsorbed to guinea pig cells from one day-old polymorphonuclear exudates gave irregular results. Four-day exudate cells from rabbits, or red blood cells from guinea pigs, sheep or rabbits, when sensitized with the derivative and injected into guinea pigs, failed to induce delayed reactions. In those instances where no delayed reactions occurred, the guinea pigs developed immediate-type Arthus reactions. Intense local bluing was observed in these animals when they were injected intravenously with Evans blue ½ hr. before skin testing. This reaction is characteristic of active cutaneous anaphylaxis[3]. The presence of antibodies to the derivative in some of these guinea pigs could be detected by passive cutaneous anaphylaxis as described by Ovary[3]. Antibodies appeared more frequently after repeated skin testing with 250 T.U. purified protein derivative of tuberculin.

Animals with characteristic tuberculin reactions did not show active cutaneous anaphylaxis. Circulating antibodies could not be detected and tests for passive cutaneous anaphylaxis were negative.

Histologically, the reactions showed the characteristic features of delayed reactions, that is, infiltration with mononuclear cells, œdema, absence of perivascular infiltrations, few polymorphonuclear leucocytes.

The absorption of soluble antigens to autologous or homologous cells might provide useful experimental models for the study of delayed sensitivity. A variety of systems with different purified protein preparations is currently being investigated.

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HUBERT BLOCH
ALBERT A. NORDIN

Department of Microbiology,
School of Medicine,
University of Pittsburgh,
Pittsburgh, Pa.
March 15.

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SOIL SCIENCE

Potassium-supplying Power of Some British Soils

British soils containing similar amounts of total potassium can vary greatly in their ability to release non-exchangeable potassium, but the reasons for this are not well understood. The non-exchangeable potassium reserves in many soils become available to plants as the exchangeable and water-soluble potassium is removed. Some soils can provide amounts of potassium adequate for crop growth during many years, but the release from reserves in others is too slow to meet crop requirements. The nature of the potassium-containing minerals and their amount and distribution of particle size are undoubtedly important; the past cropping history and the nature and amount of potassium fertilizer residues also influence the release of potassium from soils.

Continuous cropping without potassium fertilizer was used successfully in glasshouse experiments to measure releases of non-exchangeable potassium[1]; the total amount released is termed here the potassium-supplying power of the soil. For 19 different arable top soils, releases of non-exchangeable potassium to perennial ryegrass during 18 months in the glasshouse ranged from none to the equivalent of 2,000 lb. potassium per acre (Arnold, P. W., and Close, B., unpublished results). The soils, all of which contained more than 1·0 per cent total potassium, are divided in Table 1 into poor, moderate and good releasers (< 200, 200–1,000 and > 1,000 lb. potassium per acre, respectively) of non-exchangeable potassium. Only soil 19 is known to have received heavy potassium fertilization. Soils of poor and moderate potassium-supplying power were exhausted before the end of 18 months in the glasshouse, whereas the good releasers continued to provide potassium satisfactorily for grass. The clay (particles < 2μ) obtained from uncropped soils after treatment with hydrogen peroxide and dispersion in sodium hexametaphosphate was divided into parts: < 0·1, 0·1–0·3 and 0·3–2·0μ separates with a Sharples supercentrifuge. The oven-dry weights (104° C.) and the total potassium contents (hydrogen fluoride–sulphuric acid digestion, *EEL* flame photometer) of each clay separate were determined.

The potassium contents of the < 0·1μ separates and their proportions in the soils, plotted in Fig. 1, show that 17 of the 19 soils fall clearly into three categories corresponding to poor, moderate and good rates of release of potassium. High percentages of potassium in the fine clay and/or large proportions of fine clay were associated with large releases of potassium. Two soils were atypical. Soil 16, overlying glauconitic Malmstone, released considerable amounts of potassium to H-resin from silt-size particles[2]. No. 19 is a soil heavily fertilized with potassium from the Broadbalk wheat field (Plot 13); the accumulated fertilizer residues have changed the soil from a poor source of potassium, typified by soil 7 (Broadbalk Plot 11, cropped without potassium fertilizer), to a good source without increasing the potassium content of the fine clay by more than 0·2 per cent. These facts account for the good potassium-supplying powers of the two soils not being reflected in the analysis of their fine clay separates. Results for the 0·1–0·3μ clay separates were almost equally satisfactory for grouping the soils into poor, moderate and good release categories. Again, soils 16 and 19 were atypical. The potassium contents of the 0·3–2·0μ separates and their proportions in the soils, plotted in Fig. 2, showed a less well-defined relationship with the potassium-supplying power of the soils.

Table 1. Soils of Poor, Moderate and Good Potassium-supplying Power

No.	Soil series	Clay < 2μ per cent	Substratum	
1	Oak	19	Boulder Clay (non-calcareous or decalcified)	Poor potassium-supplying power, < 200 lb./acre
2	Hatfield	14	Brick-earth	
3	Thorne	24	Weald Clay	
4	Nordrach	15	Silty drift over Carboniferous Limestone	
5	Gaerwen	13	Micaceous Schist	
6	Woburn	9	Lower Greensand	
7	Batcombe	22	Clay-with-flints	
8	Hanslope	27	Chalky Boulder Clay	Moderate potassium-supplying power, 200–1,000 lb./acre
9	Hanslope	21	Chalky Boulder Clay	
10	Windsor	38	London Clay	
11	Bromyard	21	Red Marl (Downtonian)	
12	Lilleshall	20	Red Marl (Upper Coal Measures)	
13	Wicken	50	Gault Clay (resorted)	
14	Brenzett	37	Silt (marine) from Romney Marsh	Good potassium-supplying power, > 1,000 lb./acre
15	Denchworth	35	Oxford Clay	
16	Harwell	17	Malmstone (Upper Greensand, glauconitic)	
17	Evesham	35	Calcareous Lower Lias Clay	
18	Worcester	38	Red Marl (Keuper)	
19	Batcombe (potassium-fertilized annually)	21	Clay-with-flints	

Soil 7: Broadbalk, Plot 11, no potassium.
Soil 9: Saxmundham long-term experiment, Plot 4, no potassium.
Soil 19: Broadbalk, Plot 13, fertilized with potassium.

Because low potassium in the fine clay (< 0·3μ approximately) and small contents of fine clay were associated with poor releasers, fine clay particles are usually more important than coarse clay in releasing potassium in these soils. Fine distinctions between soils cannot, however, be expected, nor can it be assumed that in good releasers the coarse clay is unimportant. A detailed study of soil 9 before and after continuous cropping showed that about 40 per cent of the potassium released came from the

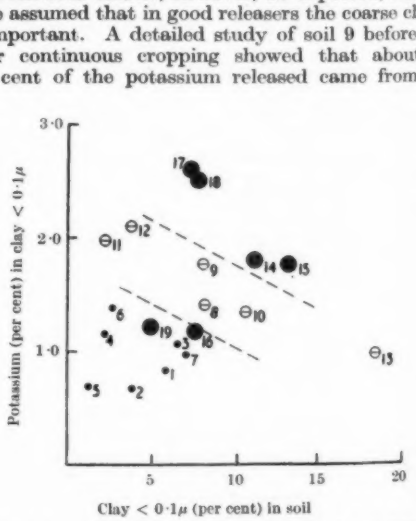

Fig. 1. Percentage and potassium content of clay < 0·1μ in relation to the potassium-supplying power of soils: •, poor; ⊖, moderate; ●, good

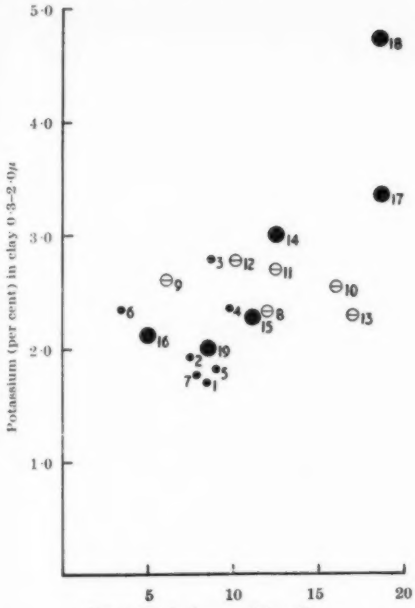

Fig. 2. Percentage and potassium content of clay 0·3–2·0μ in relation to the potassium-supplying power of soils: •, poor; ⊖, moderate; ●, good

< 0·1μ clay and 40 per cent from the 0·1–0·3μ clay, there being approximately 7 per cent of each fraction in the soil. The remaining 20 per cent was released from > 0·3μ particles which contained 75 per cent of the total potassium in the soil.

Rouse and Bertramson[3] obtained a positive correlation between the area under the 'illite' peak on X-ray spectrograms of the fine clay separates (< 0·2 and 0·2–1·0μ) and the potassium-supplying power, assessed by chemical means, of several Indiana soils. In general, however, there have been few successful attempts to relate the mineralogical composition of soils to their ability to release potassium. This is not unexpected because, as Reitemeier[4] has stated, even for the most important group of potassium-containing soil minerals, the micas and hydrous micas, their behaviour in soils is little known.

Progress in this subject needs appropriate mineralogical studies in association with simple chemical work designed to reveal trends in soil behaviour, and equally important, to recognize soils that are atypical. Without adequate physical and chemical work, mineralogical studies are likely to be unnecessarily complex, particularly on soils of diverse origin and variable age.

Thanks are due to members of the staff of the Soil Survey of England and Wales, who provided most of the soils used in the work.

P. W. Arnold

Rothamsted Experimental Station,
Harpenden,
Hertfordshire.

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STATISTICS

Analysis of Small Experiments

Considerations set out below suggest that unless we can justify certain assumptions, quantitative inferences based on the results of single small experiments may be misleading. Workers who claim they are only interested in clear-cut effects should consider carefully what they mean by this before the experiment starts. There is a danger that if a test gives a statistically significant result this will be regarded *a posteriori*, even if not *a priori*, as establishing an important effect.

Despite certain criticisms made here, small experiments have an undoubted use for suggesting further lines of research. It is also true that a series of small experiments may be more informative than one large experiment.

Significance tests and estimation procedures are commonly based on normal distribution theory, and departures from assumptions will often have more effect in small experiments than in large ones.

We consider this matter in relation to three logically distinct practical situations of common occurrence.

Situation 1. The size of the experiment is limited by the number of experimental units available. Unless we have knowledge gained from previous experience with similar material there is insufficient information in this case to postulate a particular parent distribution. Distribution free randomization tests as developed by Pitman[1] and others are appropriate, but in very small experiments they do not provide tests at a practically useful level. For example, with three replicates of each of two treatments, tests for differences between means can only be performed at certain discrete values exceeding the 10 per cent significance-level. For larger experiments this difficulty does not arise.

Situation 2. Ample potential units are available, but other factors limit the size of the experiment; the units actually used are selected on some criterion of uniformity. Normal distribution theory tests can then be justified if selection is based on standardization of a quantity about which the experimental variate is known to be normally distributed; but it is seldom that such precise knowledge is available. However, selection for uniformity usually invalidates normal distribution theory tests. If we select about average material we may obtain something like a sample from a rectangular distribution, while selection of the best available material will often lead to an upper tail sample not unlike one from an exponential distribution. Incidentally, with selection for uniformity observations need to be taken with a very high degree of accuracy, or the clustering together of values may give rise to large rounding off errors in variance estimates.

Situation 3. Ample potential units are available, other factors once more limiting the size of the experiment; the units actually used are selected at random. Normal distribution theory procedures are correct in this situation providing the variate has an approximately normal distribution over all potential units, but we can expect large standard errors. For other parent distributions sampling theory based on those distributions can be used; but this is seldom done in practice, either because of difficulty in specifying the parent distribution, or on

account of complexities in the sampling theory of all but a few distributions.

As alternatives to normal distribution theory tests are not readily available in practice, it is of interest to know something of the effects of using them when they are not theoretically justifiable. I have carried out a series of sampling trials simulating the various situations above for an experiment with three replicates of each of two treatments, there being zero treatment difference. A normal distribution theory t-test was used to detect significant differences, and too high a proportion of significant results was nearly always obtained at both the 1 per cent and 5 per cent levels when normal distribution theory assumptions did not hold. Twelve batteries, each of 200 or more tests, were performed. In only one case, corresponding to random sampling from an exponential distribution, were too few significant results obtained. When testing at the 5 per cent level the proportion of significant results was as high as 10 per cent in one battery. With another battery the proportion of results significant at the 1 per cent level was as high as 5·7 per cent. The last two cases involved samples corresponding to situations 1 and 2, respectively. When normal distribution theory assumptions held, the proportion of significant results was generally close to the test-level, for example, between 5 and 6·7 per cent when testing at the 5 per cent level.

When the alternatives to a small experiment are no experiment or a long wait, it seems clear that a decision on the course of action should not be divorced from statistical considerations associated with the logical situation. The situations discussed are not exhaustive, but cover a wide range of practical cases.

The points raised apply both to estimation and significance testing, and some of them are worthy of consideration in correlation and regression problems with few pairs of observations.

My thanks are due to Dr. S. C. Pearce for stimulating discussions on the logical status of small experiments.

P. Sprent

East Malling Research Station,
Maidstone,
Kent.

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Significance of the Difference between Two Means when the Population Variances may be Unequal

The purpose of this communication is to present a simple test applicable at the 5 per cent level to the general case of the significance of the difference between two means. If we have two samples of n_1 and n_2 variates from normal populations with the same, though unknown, scale factor, that is, $\sigma_1 = \sigma_2$, the significance of the difference between the means can be tested by the criterion:

$$t = (\bar{x}_1 - \bar{x}_2) \Big/ \sqrt{\left\{ \frac{\Sigma_1 + \Sigma_2}{n_1 + n_2 - 2} \left(\frac{1}{n_1} + \frac{1}{n_2} \right) \right\}}$$

where Σ_1 and Σ_2 represent the sums of squares from the respective means and t is referred to Student's integral with $n_1 + n_2 - 2$ degrees of freedom. If the scale factors are unequal the use of this criterion involves a bias[1] which is small if $n_1 = n_2$ but which can be large if $n_1 \neq n_2$. When the relative scale factor of the two populations is known, appropriate weighting of the sums of squares gives an exact solution. In the case where the relative scale factor is unknown, Fisher[2,3] advocates the use of a different criterion, Behrens' d [4], which can be expressed as:

$$d = (\bar{x}_1 - \bar{x}_2) \Big/ \sqrt{\left\{ \frac{\Sigma_1}{n_1(n_1 - 1)} + \frac{\Sigma_2}{n_2(n_2 - 1)} \right\}}$$

and tables[5,6] are available for the 5 per cent and 1 per cent points of this distribution for a lattice of values of n_1 and n_2 both greater than 6.

In a previous communication[7] it was pointed out that when Student's t is standardized by dividing it by its own standard deviation, the variability of the tails with the number of degrees of freedom is greatly reduced and in particular the 5 per cent values of standardized t, denoted t_s, for 4 or more degrees of freedom all lie within the limits 1·96 and 2·0. With reference to the present problem:

$$t_s = (\bar{x}_1 - \bar{x}_2) \Big/ \sqrt{\left\{ \frac{\Sigma_1 + \Sigma_2}{n_1 + n_2 - 4} \left(\frac{1}{n_1} + \frac{1}{n_2} \right) \right\}}$$

Behrens' d can be adjusted in a similar way giving:

$$d_s = (\bar{x}_1 - \bar{x}_2) \Big/ \sqrt{\left\{ \frac{\Sigma_1}{n_1(n_1 - 3)} + \frac{\Sigma_2}{n_2(n_2 - 3)} \right\}}$$

From the relationship between Behrens' d and its adjusted value d_s, implicit in the above formulæ, the value of d_s corresponding to each value of d in the 5 per cent table was calculated with the remarkable result that all these values of d_s lie within the range 1·96 to 2·0 whereas d itself ranges from 1·960 to 2·447. Hence, provided that n_1 and n_2 are both greater than 6, if $d_s \geqslant 2$ the difference between the means is significant at the 5 per cent level; if $d_s < 1{\cdot}96$, the difference is not significant. The formula was suggested by Welch but he did not give numerical values.

When $n_1 = n_2 = n$, say, the formulæ for t and d are identical but the numerical values for a given level of significance are different; d is more stringent. The corresponding adjusted criteria are:

$$t_s = (\bar{x}_1 - \bar{x}_2) \Big/ \sqrt{\left\{ \frac{\Sigma_1 + \Sigma_2}{n(n - 2)} \right\}}$$

and

$$d_s = (\bar{x}_1 - \bar{x}_2) \Big/ \sqrt{\left\{ \frac{\Sigma_1 + \Sigma_2}{n(n - 3)} \right\}}$$

Both lie between 1·96 and 2·0 at the 5 per cent point for $n > 6$. The factor $n - 3$ compared with $n - 2$ reflects the absence of information about the relative scale factors of the two populations.

John B. de V. Weir

University of Glasgow,
Glasgow, W.2.

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APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT (honours graduate) IN THE DEPARTMENT OF BOTANY—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (August 5).

ASSISTANT LECTURER IN PURE MATHEMATICS—The Registrar, The University, Sheffield (August 6).

UNESTABLISHED ASSISTANT EXPERIMENTAL OFFICER (with a degree in agriculture or pure science with main subject zoology) IN THE DEPARTMENT OF AGRICULTURE AND FISHERIES FOR SCOTLAND, PERTH—The Ministry of Labour, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting G265/OA (August 6).

RESEARCH ASSISTANTS (3) IN THE DEPARTMENT OF METALLURGY, County Technical College, Wednesbury, to work on one of a variety of research projects, several of these being within the field of atomic energy—The Director of Education (Staffing), County Education Offices, Stafford (August 9).

ASSISTANT (with special interest in pig husbandry) IN ANIMAL HUSBANDRY—The Secretary, The University, Aberdeen (August 12).

RESEARCH STUDENTS (with a good honours degree in chemistry or biochemistry) IN THE BIOCHEMISTRY DIVISION OF THE PHYSIOLOGY DEPARTMENT, for investigation on the chemistry and biochemistry of lipids, leading to a M.Sc. or Ph.D.—Dr. C. Long, Biochemistry Division, Physiology Department, Royal College of Surgeons of England, Lincoln's Inn Fields, London, W.C.2 (August 13).

TEMPORARY ASSISTANT LECTURER IN PURE MATHEMATICS—The Registrar, University College, Singleton Park, Swansea (August 13).

LECTURER IN THE DEPARTMENT OF METALLURGY for duties primarily concerned with teaching and research in diffraction metallography with particular reference to X-ray work—The Registrar, The University, Liverpool, quoting Ref. CV/T (August 15).

LECTURER OR ASSISTANT LECTURER IN ZOOLOGY with special reference to vertebrate zoology or genetics, at the University of Malaya (Singapore Division)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 15).

LECTURERS OR ASSISTANT LECTURERS (3) IN THE DEPARTMENT OF GEOGRAPHY, University of Malaya, Kuala Lumpur—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Kuala Lumpur and London, August 15).

LIBRARIAN (man or woman graduate with a library qualification and preferably a scientific background)—The Secretary, Royal Veterinary College (University of London), Royal College Street, London, N.W.1 (August 15).

NEUROPHYSIOLOGIST (with Ph.D. or equivalent) IN THE DEPARTMENT OF ZOOLOGY for research on central nervous physiology of the Lepidoptera—The Secretary, University College, Gower Street, London, W.C.1 (August 15).

LECTURER OR VISITING LECTURER IN GEOGRAPHY at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand and London, August 19).

FOUNDATION CHAIR OF PHILOSOPHY in Monash University, Clayton, Victoria, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, August 22).

JUNIOR LECTURER IN ZOOLOGY—The Registrar, Trinity College, Dublin (August 22).

SENIOR LECTURER IN CROP HUSBANDRY—The Principal, Seale-Hayne Agricultural College, Newton Abbot, Devon (August 22).

ASSISTANT LECTURER IN CHEMICAL PATHOLOGY—The Dean, St. Thomas's Hospital Medical School, London, S.E.1 (August 26).

SENIOR LECTURER, and a LECTURER IN SOIL SCIENCE at the University College of Ghana—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (August 30).

KEEPER (graduate) OF THE SCIENCE BOOKS in the University Library—The Librarian, Durham University Library, Palace Green, Durham (September 3).

LECTURER IN THE DEPARTMENT OF GEOLOGY—The Secretary, The University, Aberdeen (September 3).

ASSOCIATE PROFESSOR (with special interests in ballistics) IN THE DEPARTMENT OF MATHEMATICS; an ASSOCIATE PROFESSOR (with special interests in applied mechanics, including fluid dynamics) IN THE DEPARTMENT OF CIVIL AND MECHANICAL ENGINEERING; and an ASSOCIATE PROFESSOR (with special interests in applied thermodynamics) IN THE DEPARTMENT OF CIVIL AND MECHANICAL ENGINEERING, Royal Military College of Science, Shrivenham—Civil Service Commission, 17 North Audley Street, London, W.1, quoting Ref. S/5156/60 (September 5).

LECTURER IN PSYCHOLOGY at the University of Tasmania—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, September 7).

LECTURER OR SENIOR LECTURER IN FLUID DYNAMICS at the University of Tasmania—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, September 7).

LECTURER/SENIOR LECTURER (preferably with experience in medical bacteriology) IN BACTERIOLOGY at the University of Sydney—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, September 9).

UNIVERSITY LIBRARIAN (university graduate with appropriate professional experience)—The Registrar, The University, Liverpool (September 10).

SENIOR LECTURER (with a degree in dairying or a degree in pure science or agriculture with a diploma in dairying or in dairy technology) IN DAIRYING—The Secretary, West of Scotland Agricultural College, 6 Blythswood Square, Glasgow, C.2 (September 14).

LECTURER/SENIOR LECTURER (qualified in grassland management) IN ANIMAL HUSBANDRY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, September 15).

SENIOR LECTURER and a LECTURER (with an honours degree, preferably in chemical engineering, with research and/or industrial experience) IN CHEMICAL ENGINEERING at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, September 15).

SENIOR LECTURER IN CLINICAL BIOCHEMISTRY at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia and London, September 30).

CHAIR OF CHEMISTRY at the School of Pharmacy—The Academic Registrar, University of London, Senate House, London, W.C.1 (October 14).

AGRICULTURAL OFFICER (Tobacco Research) (with an honours degree in natural science (botany), or a degree in horticulture, with suitable postgraduate training or experience), in Nyasaland for experimental work on tobacco including agronomy trials and plant breeding—The Director of Recruitment, Colonial Office, London, S.W.1, quoting Ref. BCD.63/4/04/T.

AGRONOMIST (Livestock), (with a good honours degree in agriculture and postgraduate experience or training in animal genetics) in Uganda, to take charge of a large breeding scheme designed to produce improved beef cattle utilizing local and imported stock—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/9/038.

ASSISTANT LECTURER or LECTURER (with qualifications in zoology with biochemistry or physiology)—The Principal, Derby Training College, Uttoxeter New Road, Derby.

CENTRAL ELECTRICITY BOARD RESEARCH FELLOW (with a good degree in electrical engineering or physics of a British university or an equivalent qualification, and preferably experience of vacuum electronics) IN THE DEPARTMENT OF ELECTRICAL ENGINEERING for work on thermo-electron engines—The Secretary, University College, Gower Street, London, W.C.1.

LECTURER IN TROPICAL HYGIENE—The Dean, London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1.

LECTURER or ASSISTANT LECTURER IN MATHEMATICS at Makerere College (University College of East Africa)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1.

LECTURER (with an honours degree in chemical engineering or Corporate Member of the Institute of Chemical Engineers) IN CHEMICAL ENGINEERING—The Registrar, The University, Nottingham.

LECTURERS (2) IN ZOOLOGY, one to teach histology and microtechnique, and the other to teach ornithology (and if possible animal behaviour)—McGill University, Montreal, Quebec, Canada.

PROFESSOR or ASSOCIATE PROFESSOR (male graduate of a British university with appropriate teaching experience) OF BIOLOGY at Nanyang University, Singapore—The Director, Recruitment Department, British Council, 65 Davies Street, London, W.1, quoting Ref. N.11 T(60).

SENIOR DEMONSTRATOR IN THE DEPARTMENT OF PHYSICS—The Secretary, Birkbeck College (University of London), Malet Street, London, W.C.1.

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